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RELATION OF NITROGEN TO YIELD OF SUGAR-BEET SEED AND TO ACCOMPANYING CHANGES IN COMPOSITION OF THE ROOTS [1]

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INTRODUCTION

The method of growing sugar-beet seed by overwintering the plants in the field has come into widespread use in the United States during the past decade. Overpeck (*11*),[4] 1928, was the first to report observations showing that sugar beets planted in the early fall in southern New Mexico would survive the winter and produce a satisfactory seed crop during the following summer. Since that time the growing of seed has expanded rapidly until, at the present time, commercial crops are being grown by this method in New Mexico, Utah, California, Nevada, Texas, and Arizona. Recent developments in cultural practices for the crop have been reported by Overpeck and Elcock (*4*, *12*).

From results with the sugar-beet seed crop in various areas, it appears that soils of good tilth and more than average fertility are desirable for growing the crop. Heavy applications of barnyard manure are employed in the Mesilla Valley of New Mexico and in southern Utah. In areas where the industry has become established, nitrogenous fertilizers, chiefly as spring applications, are used. In southern Utah many farmers also apply phosphate, usually at planting time. Experiments [5] are being carried on to determine the best fertilizer practices for the different areas now engaged in sugar-beet seed production. Because of the emphasis upon fertility investigations and the strong interest of growers in methods of stabilizing and improving yields of seed while maintaining high quality, studies that seek to establish a sound physiological basis for the fertilizing practice are needed and should have important directive influence.

The experiments reported in this paper deal chiefly with the function of nitrogenous fertilizers in the fructification stage of the sugar beet. Study has been made of physiological processes of the sugar beet, special attention being given the changes in the carbohydrate and nitrogen content of the plants during the period of fructification.

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[2] The writer wishes to express his appreciation of the suggestions and criticisms given by Eubanks Carsner, senior pathologist, and G. H. Coons, principal pathologist, Division of Sugar Plant Investigations, in connection with the preparation of the manuscript.

[3] Resigned Mar. 15, 1936.

[4] Reference is made by number (italic) to Literature Cited, p. 563.

[5] Cooperative experiments of the Divisions of Sugar Plant Investigations and Soil Fertility Investigations.

The accumulation by the sugar beet of a large reserve of sucrose during the period of vegetative development is a distinctive characteristic of this plant. The functioning of this accumulation of carbohydrate reserve in the life cycle of the plant has received little attention. It has commonly been assumed that at least a part of the sucrose stored in the taproot during the first season of growth was drawn upon for the development of the seed-producing structures and seed the following year; little is known about the effect of various environmental factors in conditioning such utilization.

In studying the influence of environmental factors on seed production in the sugar beet, an approach was made by determining the effect of nitrogen fertilization and other factors upon the seasonal changes in carbohydrate and nitrogen content in the roots of the sugar beet, and, where possible, these changes were correlated with yields of seed obtained.

FIELD STUDIES

METHODS

Most of the results reported in this paper were obtained from plants grown on experimental plots in the sugar-beet seed growing area near St. George, Utah. A few samples from plots located at State College, N. Mex. were tested in 1934.

In all of these tests seed of a curly top-resistant variety (U. S. 1) was planted at a rate of 20 pounds per acre. In 1931 the seed was planted on September 10. In 1932 plantings were made on August 23 and September 15. The treatments were arranged in randomized blocks and replicated either four or five times.

The quantities of fertilizers applied were in excess of those ordinarily recommended to commercial growers, the object being to produce strong differences in the physiological responses of the plants. At the time of seeding in 1931 the entire experimental field was treated with 45-percent treble superphosphate at the rate of 175 pounds per acre. In 1932 the rate of application was 125 pounds per acre. The phosphate fertilizer was applied in the row approximately 1 inch below the seed.

The experiment in 1931 was set up with the following schedule of treatments, ammonium sulphate being used as the source of nitrogen:

Treatment no. 1.—750 pounds per acre, September 25, 1931.
Treatment no. 2.—375 pounds per acre, September 25, 1931; 375 pounds per acre, March 2, 1932.
Treatment no. 3.—750 pounds per acre, March 2, 1932.
Treatment no. 4.—None.

The experiments of 1932–33 were designed to furnish further evidence concerning the effects of applications of a nitrogeneous fertilizer on seed yield and on the chemical composition of the roots, and in addition to show the effects of date of planting and spacing. Each plot consisted of eight rows 22 inches apart and 45 feet long. The thinning and fertilizer treatments were carried out according to the schedule shown as table 1.

The center two rows of each plot were harvested to obtain data on seed yield.

TABLE 1.—*Schedule of thinning and fertilizer treatments for the experiments of 1932–33*

Treatment no.	Date planted	Date thinned [1]	Date fertilized	Ammonium sulphate per acre
				Pounds
1	Aug. 23, 1932	Sept. 25, 1932	Sept. 25, 1932	500
			Feb. 27, 1933	500
2	do	do		0
3	do	Unthinned	Sept. 25, 1932	500
			Feb. 27, 1933	500
4	do	do		0
5	Sept. 15, 1932	Oct. 22, 1932	Oct. 22, 1932	500
			Feb. 27, 1933	500
6	do	do		0
7	do	Unthinned	Oct. 22, 1932	500
			Feb. 27, 1933	500
8	do	do		0

[1] In the thinned plots the plants were spaced to 12-inch intervals; in the unthinned plots the original stand was left.

RESULTS

YIELD OF SEED

Applications of nitrogenous fertilizer were responsible for large and highly significant [6] increases in the yield of sugar-beet seed during two seasons. Although the quantities of fertilizer applied were in excess of those ordinarily recommended to commercial growers, they were effective in producing yield increases that were correlated with certain physiological responses of the plants. The pertinent yield data are given in tables 2 and 3.

Taking all the data into consideration, the effect of nitrogenous fertilizer was more pronounced than that of any other phase of treatment. In 1931–32 the plots in which ammonium sulphate was applied in the fall or early spring yielded 35 percent more seed than the unfertilized plots, but the different dates of application resulted in no significant difference in yield. Again in 1932–33 the nitrogenous fertilizer produced marked increases in the yield of seed, but in this season the effect was modified to a considerable extent by the date of planting. In the August 23 planting the application of ammonium sulphate on thinned and unthinned plots resulted in increased yields of 30 and 45 percent, respectively, while in the September 15 planting the same treatments resulted in gains of only 22 and 15 percent over the unfertilized plots.

TABLE 2.—*Effect of nitrogen on the yield and percentage of germination of sugar-beet seed, St. George, Utah, 1931–32*

Treatment no.	Season when ammonium sulphate was applied	Yield per acre	Average germination
		Pounds [1]	*Percent* [2]
1	Fall	3,545	74.0
2	Fall and spring	3,642	75.7
3	Spring	3,790	80.3
4	None	2,719	76.2

[1] Difference required for significance, 285 pounds.
[2] Differences not statistically significant.

[6] The yield data have been analyzed according to the analysis of variance method (*5*).

TABLE 3.—*Effect of date of planting, thinning, and nitrogen fertilization on yield, percentage of germination, and weight of sugar-beet seed, St. George, Utah, 1932–33*

Treatment and date of planting	Yield of seed per acre	Germination of seed	Seed balls per gram	Treatment and date of planting	Yield of seed per acre	Germination of seed	Seed balls per gram
Unthinned:				Thinned:			
No nitrogen:	*Pounds* [1]	*Percent* [2]	*Number* [2]	No nitrogen:	*Pounds* [1]	*Percent* [2]	*Number* [2]
Aug. 23	2,196	54	58.5	Aug. 23	2,625	55	58.8
Sept. 15	2,302	50	61.4	Sept. 15	2,278	56	53.5
Nitrogen:				Nitrogen:			
Aug. 23	3,189	56	59.5	Aug. 23	3,412	56	59.5
Sept. 15	2,649	44	63.3	Sept. 15	2,782	52	60.2

[1] Difference required for significance, 502.2 pounds.
[2] Differences not statistically significant.

When the effects of date of planting are considered independently of other treatments, in general the earlier planting resulted in decidedly higher seed yields. This is in accord with the results reported by Elcock and Overpeck (*4*) in New Mexico.

Thinning the plants so that they stood singly in the row increased the yield of seed approximately 19.5 percent in the case of the beets that were planted early and were not fertilized. In all other cases where direct comparisons can be made, the thinning treatment had no marked effect on the yield of seed. Since these results are for a single season they indicate only that under the conditions of this test the space available to each plant was not a factor in influencing the yield of seed, save where soil fertility was too low to permit the full development of large numbers of plants in the unthinned stands.

Quality of Seed

In the season of 1931–32 the heavy applications of ammonium sulphate had little noticeable effect on the quality of the seed produced (table 2). The average germination of the seed from the fertilized plots was 76.7 percent while that of the checks was 76.2 percent.

The following year all of the seed was markedly lower in germination but only in the fertilized, unthinned series of the late planting was there any evidence that viability of the seed was altered by a particular treatment. In this case the germinative power of the seed dropped so markedly below the average for the other treatments as to suggest that caution is necessary in applying nitrogenous fertilizers to beets planted late and left unthinned.

The size of the seed appeared to be influenced somewhat by the fertilizer treatments (table 3). Although all of the seed from the 1932–33 crop was small, i. e., each gram contained more than 51 seed balls, the ammonium sulphate treatments caused small but consistent decreases in the size of the seed balls. These differences, however, are not of sufficient magnitude to be considered detrimental to the quality of the seed.

Top Development

The characteristic effects of nitrogen on top growth were noticeable soon after the ammonium sulphate was applied to the beets in the fall. During the vegetative period that preceded winter dormancy, the fertilizer caused an increase in leaf surface that was apparently related to an increase in the amount of sucrose stored in the roots (fig. 1).

More important, however, was the effect of the fertilizer on the reproductive structures developed during the following spring and early summer. The seedstalks of the fertilized plants were taller and sturdier and were characterized by much longer seed branches than were the unfertilized plants. This latter effect is significant, since the length of the seed branches to a large extent determines the number of flowers and fruits which can be formed.

The onset of nitrogen deficiency in the unfertilized plants occurred early in May and was marked by a yellow color of the tops. As the nitrogen deficiency became more severe the elongation of the seed branches ceased and the result was a much-abbreviated period of flower production. In contrast, the fertilized plants remained green for a much longer period and the length of the seed branches and the number of flowers and fruits formed were correspondingly greater. This phase of top development was at least partly responsible for the highly significant (*16, p. 62*) correlation of +0.609, which was obtained when the green weights of tops from 26 plots were correlated with the seed yields from the same plots.

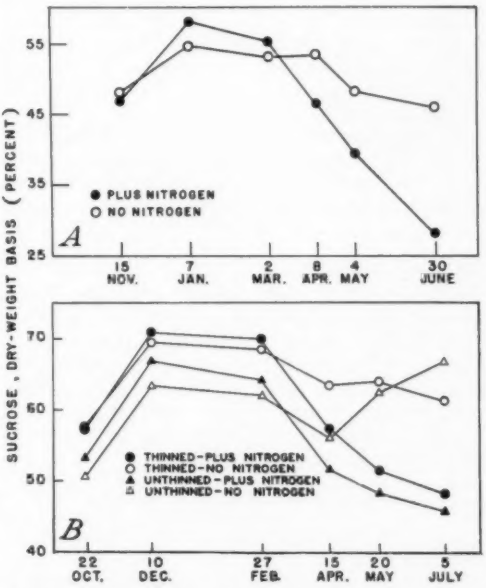

FIGURE 1.—Seasonal changes in the percentage of sucrose in the roots of sugar beets overwintered in the field: *A*, 1931–32; *B*, 1932–33.

Corroborative evidence in this connection is afforded by observations made upon effects of late heavy applications of ammonium sulphate in commercial fields at St. George in 1933. In these tests it was observed that while nitrogen applied during the flowering period increased the yield of seed in all cases, the increase was much greater in fairly good fields than in poor fields. The reason for this seemed to be that the plants in the poor fields had been definitely checked by nitrogen deficiency before the nitrogen was applied, with the result that the original growing points did not resume development. The production of new lateral branches was stimulated but these, starting late, did not mature seed by the time of harvest.

ROOT DEVELOPMENT

The data presented in figure 2 and table 4 show that the nitrogenous fertilizer had little effect on the size of the roots developed during the

fall and winter. When sampled on February 27, the only difference in size of roots occurred between the thinned and unthinned plots.

After seedstalk development began in the spring, the size of the roots in the unthinned plots changed only slightly. In the thinned plots fertilized with ammonium sulphate the roots increased slowly in size between April 15 and harvest time, but where no fertilizer was added the roots made a pronounced growth during this period. This condition led to a rather striking difference in the root-top ratios at harvest time and indicates that the plants receiving no nitrogen were storing materials in the roots during the period when the fertilized plants were using similar materials for top growth and seed production. Further evidence that this occurred will be brought out when the carbohydrate analyses are discussed. The fact that the plants receiving no fertilizer in the unthinned series did not show an increase in root size comparable to that in the thinned series seemed to be due to the intense competition for soil nitrogen and moisture in the plots where the stand was thick. The foliage of these plants became yellow early in May and apparently the small supply of soil nitrogen was used to mature the seed, other growth being reduced to a minimum.

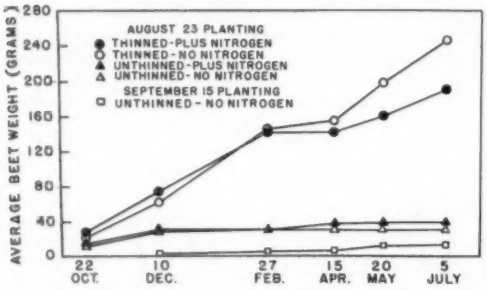

FIGURE 2.—Change in weight of roots of sugar beets overwintered in field (1932–33).

TABLE 4.—*Effect of date of planting, thinning, and nitrogen fertilization on weight of roots and tops at harvest, St. George, Utah, 1932–33*

Treatment and date of planting	Green weight of tops per plot	Average weight per root	Treatment and date of planting	Green weight of tops per plot	Average weight per root
Unthinned:			Thinned:		
No nitrogen:	*Kilograms*	*Grams*	No nitrogen:	*Kilograms*	*Grams*
Aug. 23	60.0	25.5	Aug. 23	79.3	245.6
Sept. 15	61.7	11.3	Sept. 15	43.1	157.0
Nitrogen:			Nitrogen:		
Aug. 23	95.4	36.8	Aug. 23	107.4	189.5
Sept. 15	82.4	14.3	Sept. 15	68.3	98.1

CHEMICAL STUDIES

METHODS

Chemical analyses were made of composite samples from the various treatments taken at intervals between November 15, 1931, and June 30, 1932, and again between October 22, 1932, and July 5, 1933. The plants were usually dug early in the morning, and approximately 24 hours elapsed before they reached the laboratory. In the laboratory, the roots were washed free from soil and dried lightly with

towels, and the crowns and roots were separated by topping the beets just below the lowest leaf scar. The tissues were then minced and mixed in a wooden chopping bowl and samples were taken for moisture determination. At the same time a 30- to 40-g sample for the carbohydrate determinations was preserved by boiling for 30 minutes in neutral 80-percent alcohol. When necessary the sample was adjusted to neutrality (litmus) after boiling. The rest of the minced tissue was placed in fruit jars, sealed, and taken immediately to a cold-storage room where it was held constantly at 12.5° F. until sampled for the determination of nitrogen fractions and total nitrogen.

Carbohydrate Analysis

The preserved material was prepared for analysis by extracting with 80-percent alcohol in Soxhlet extractors until a negative Molisch (*9, p. 942*) test was obtained. All of the alcoholic filtrates were combined, and the alcohol was removed by heating on a water bath at approximately 75° C. Several small portions of water were added, when the solution became sirupy, until the removal of the alcohol was complete. The water extract was then made up nearly to volume in a 250-cc flask and cleared by the addition of 2 cc of neutral lead acetate solution (specific gravity 1.25). The sample was then diluted to volume, mixed thoroughly, and filtered through a dry paper into a beaker containing 0.75 g of anhydrous potassium oxalate. The sugar determinations were made on aliquots of this filtrate.

Direct reducing sugars were determined in a 50-cc aliquot of this filtrate. Another aliquot was hydrolyzed at room temperature by the method outlined by the Association of Official Agricultural Chemists (*2, p. 187*), before reduction. The difference in reducing power of the solution before and after hydrolysis is considered to be a measure of the sucrose present.[7] The Shaffer-Hartman (*15*) modification of the Munson-Walker method was used to determine the reducing power of all sugar solutions, and all of the results for the different carbohydrates are expressed as percentages of glucose.

The insoluble residue remaining after the extraction with alcohol was analyzed, the method outlined by Potter and Phillips (*13*) being used. The quantity of starch and dextrins present was so small that it is not reported, but these substances were always removed in the routine analysis before the insoluble residue was hydrolyzed with dilute hydrochloric acid for the determinations of other polysaccharides.

Nitrogen Analysis

The frozen tissue preserved for the determination of nitrogen fractions and total nitrogen was ground in a chilled Nixtamal mill. The ground tissue was then mixed thoroughly, a 100-g sample was taken for extraction with water, and the fractionation into soluble, coagulable,[8] and unextracted nitrogen was carried out with slight modification of the methods outlined by Nightingale et al. (*10*). Total nitrogen was determined on a sample of dried tissue. The Kjeldahl method, modified to include nitrates (*14*), was employed. Nitrate nitrogen was determined on an aliquot of the extract from

[7] No correction has been made for small amounts of raffinose that were included with the sucrose.

[8] The nitrogen content of the coagulum was determined, but no attempt is made to interpret the variations in this fraction because of the differences known to result from different amounts of grinding.

which alcohol had been removed. The removal of ammonia and amide nitrogen, and the subsequent reduction of nitrates and nitrites with finely ground Devarda's alloy, was carried out according to the method outlined by Phillips et al. (*1, p. 209*).

PLANT UTILIZATION OF NITROGEN

Seasonal changes in various fractions of nitrogen are presented in tables 5 and 6, and changes in total nitrogen are represented graphically in figure 3. All the results of analyses were calculated as percentages of fresh weight and as percentages of dry weight; but, because of the marked similarity of the trends shown by the two methods of representation, only the latter are used here. Insofar as the experiments of the two seasons duplicated each other, the analytical results from similar treatments are in close agreement. The data from the unthinned treatments of 1931–32 can be compared with the data from the unthinned treatments of the following season.

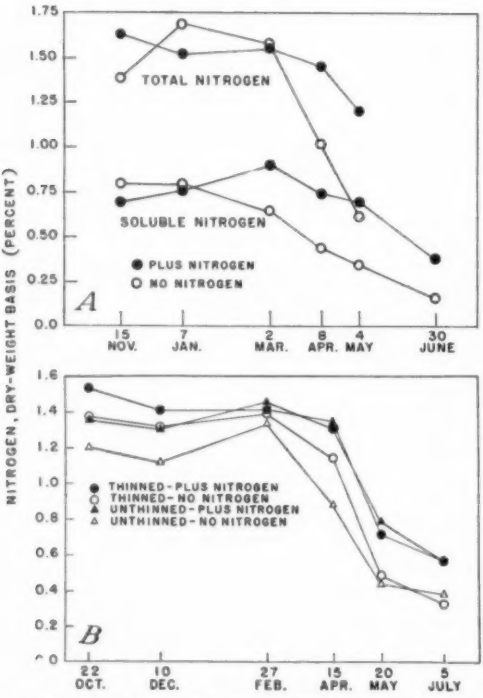

FIGURE 3.—Seasonal changes in the percentage of nitrogen in the roots of sugar beets overwintered in the field: *A*, Total and soluble nitrogen, 1931-32; *B*, total nitrogen, 1932-33.

TOTAL NITROGEN

The percentage of total nitrogen in the roots from all of the treatments showed some variations during the fall and winter seasons. In general, the fertilized plants were slightly lower in total nitrogen in the spring than they were in the fall, while the reverse was true of the unfertilized plants. In both years, however, the proportion of total nitrogen was very similar in the samples from all treatments taken just prior to seedstalk initiation (Mar. 2, 1932, and Feb. 27, 1933). There was no indication in these experiments that the application of ammonium sulphate in the fall resulted in any accumulation of nitrogen in the taproots of the fertilized plants.

After seedstalk growth began in the spring, nitrogen was withdrawn rather rapidly from the taproots. During this time the effect of the fertilizer was shown by the divergence in the total nitrogen curves

(fig. 3), the fertilized roots maintaining a level of total nitrogen consistently higher than that of the unfertilized roots. This relationship continued until harvest time, although in both cases from 60 to 70 percent of the nitrogen had been withdrawn by the end of the fruiting period.

TABLE 5.—*Seasonal changes in various carbohydrate and nitrogen fractions in the roots of beets overwintered in the field, St. George, Utah, 1931–32*

[Results expressed on dry-weight basis]

Date of sampling	Treatment	Carbohydrate fraction		Nitrogen fraction				
		Sucrose	Reducing sugars	Total nitrogen	Soluble nitrogen	Coagulable nitrogen	Protein nitrogen	NO_3 nitrogen
1931		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Nov. 15	Unfertilized	47.74	1.64	1.4018	0.8088	0.4079	0.5930	0.0432
	Plus nitrogen	46.79	1.85	1.6411	.6825	.4367	.9586	.1669
1932								
Jan. 7	Unfertilized	54.67	1.46	1.6893	.7907	.3904	.8986	.0297
	Plus nitrogen	58.13	2.25	1.5193	.7610	.3144	.7583	.1047
Mar. 2	Unfertilized	53.05	1.37	1.5766	.6439	.3033	.9327	.0884
	Plus nitrogen	55.20	1.87	1.5632	.9002	.2566	.6630	.1398
Apr. 8	Unfertilized	53.14	1.28	1.0094	.4429	.2616	.5665	.0766
	Plus nitrogen	46.54	1.11	1.4675	.7375	.2517	.7300	.2785
May 4	Unfertilized	48.15	.99	.6195	.3437	.0978	.2758	.0961
	Plus nitrogen	39.54	1.23	1.1909	.7026	.1434	.4883	.2621
June 30	Unfertilized	45.95	.89	----------	.1675	----------	----------	.0000
	Plus nitrogen	28.66	1.64	----------	.3812	----------	----------	.2090

TABLE 6.—*Seasonal changes in percentages of various nitrogen fractions in roots of sugar beets [1] overwintered in the field, St. George, Utah, 1932–33*

[Results expressed on dry-weight basis]

Date of sampling	Treatment	Nitrogen fraction			
		Total nitrogen	Soluble nitrogen	Insoluble protein nitrogen	NO_3 nitrogen
1932		*Percent*	*Percent*	*Percent*	*Percent*
Oct. 22	Unthinned, no nitrogen	1.207	0.686	0.521	0.103
	Unthinned, nitrogen	1.359	.778	.581	.140
	Thinned, nitrogen	1.539	.875	.664	.112
	Thinned, no nitrogen	1.377	.819	.558	.068
Dec. 10	Unthinned, no nitrogen	1.176	.677	.499	.094
	Unthinned, nitrogen	1.306	.803	.503	.111
	Thinned, nitrogen	1.412	.931	.481	.107
	Thinned, no nitrogen	1.318	.832	.486	.080
1933					
Feb. 27	Unthinned, no nitrogen	1.332	.783	.549	.100
	Unthinned, nitrogen	1.457	.991	.466	.135
	Thinned, nitrogen	1.415	1.006	.409	.129
	Thinned, no nitrogen	1.390	.906	.484	.089
Apr. 15	Unthinned, no nitrogen	.881	.478	.403	.075
	Unthinned, nitrogen	1.309	.763	.546	.244
	Thinned, nitrogen	1.268	.732	.536	.263
	Thinned, no nitrogen	1.151	.535	.616	.169
May 20	Unthinned, no nitrogen	.443	.140	.303	.013
	Unthinned, nitrogen	.788	.392	.396	.165
	Thinned, nitrogen	.720	.351	.369	.143
	Thinned, no nitrogen	.497	.169	.328	.015
July 5	Unthinned, no nitrogen	.387	.127	.260	.000
	Unthinned, nitrogen	.573	.366	.207	.114
	Thinned, nitrogen	.573	.338	.235	.098
	Thinned, no nitrogen	.339	.099	.206	.000

[1] Planted Aug. 23, 1932.

Soluble and Insoluble Nitrogen

The soluble nitrogen in the roots tended to reach highest levels about the time growth of the plants was resumed in the spring. At that time the insoluble nitrogen, calculated as percentage of total nitrogen, was low, indicating that some hydrolysis of proteins occurred before seedstalk development started. The fact that this relation between soluble and insoluble nitrogen was much more pronounced in the fertilized plants than it was in those not fertilized indicates that the fall applications of ammonium sulphate had some influence on the form of nitrogen in the roots, even though they did not affect the total amount of nitrogen accumulated. On March 2, 1932, approximately 58 percent of the nitrogen in the fertilized roots was soluble as compared with 41 percent in the unfertilized roots. A similar relationship was shown by the samples taken on February 27, 1933.

The tendency of the plants receiving nitrogenous fertilizer to attain a high ratio of soluble to insoluble nitrogen at this stage of development may be related to the earlier and more vigorous seedstalk growth that has been observed in both field and greenhouse experiments where abundant nitrogen has been supplied to the plants during the vegetative period.

During April, May, and June, when the fruiting structures were being developed, the percentage of insoluble nitrogen decreased at about the same rate in the roots from all treatments. Soluble nitrogen decreased at the same time, but the decrease was more rapid and reached lower levels in the unfertilized plants than it did in those receiving ammonium sulphate.

Nitrate Nitrogen

Insofar as the percentage of nitrate nitrogen found in the roots at the various dates can be considered indicative of the availability of nitrogen in the soil, these data show that the unfertilized plants had reduced the supply of soil nitrogen to a very low point fully 6 weeks before the seed was harvested. In the fertilized plants determinable amounts of nitrate nitrogen were found in the roots until the end of the season.

Plant Utilization of Carbohydrates

Sucrose

Seasonal changes in the sucrose percentage of the roots, presented in tables 5 and 7 and represented graphically in figure 1, show that sucrose reached a maximum in early winter and declined only slightly between that time and early spring. During this period, however, the treatments caused definite differences in the percentage of sucrose in the roots. Where the plants were spaced in the row, the roots were markedly higher in sucrose than were the roots in the corresponding unthinned plots. The fall applications of ammonium sulphate also resulted in a definite increase in sucrose accumulation in the roots of the plants receiving the fertilizer. In general, these differences remained constant until spring.

After seedstalk development began in the spring, the early effects on sucrose percentage caused by spacing were overshadowed by the pronounced effect of the spring application of nitrogenous fertilizer. In

general, the sucrose percentage of all the roots decreased rapidly in the interval between the March and April samplings, but after the middle of April there was a striking divergence in the sucrose curves. The roots of the plants that received ammonium sulphate in the spring underwent a consistent decrease in sucrose until the seed was harvested, while the roots of the unfertilized plants showed only slight decreases or actual gains in the percentage of sucrose. The rather contrasting behavior of the thinned and unthinned plants that received no fertilizer in the spring is probably more apparent than real, since the root weight of the thinned plants increased rapidly during May and June, whereas the unthinned plants maintained relatively constant weight during this period (fig. 2). It is probable that the thinned plants accumulated sucrose in much the same way that the unthinned plants did, but the increase is not apparent when the results are calculated on the dry-weight basis.

This storage of sucrose by plants not receiving applications of ammonium sulphate is interpreted as having very significant relationships to the yield and will be discussed more fully later.

Reducing Sugars

The changes in reducing sugars presented in tables 5 and 7 do not show definite trends in all cases, but in general the plants receiving nitrogen gave higher values for reducing sugars throughout the season than did the unfertilized plants. The difference was especially marked during the fructification stages of late spring and early summer. The significance of these trends is not clear, but a somewhat similar relationship found in sugarcane (*3*) has been attributed to the greater hydration of tissue that results from nitrogen fertilization.

Table 7.—*Seasonal changes in percentages of various carbohydrate fractions in roots of sugar beets* [1] *overwintered in the field, St. George, Utah, 1932–33*

[Results expressed on dry-weight basis]

Date of sampling	Treatment	Carbohydrate fraction		
		Sucrose	Reducing sugars	Acid hydrolyzable polysaccharides
		Percent	*Percent*	*Percent*
1932				
Oct. 22	Unthinned, no nitrogen	50.64	1.14	23.86
	Unthinned, nitrogen	53.33	.97	23.15
	Thinned, nitrogen	57.24	.95	22.51
	Thinned, no nitrogen	57.66	.86	23.78
Dec. 10	Unthinned, no nitrogen	63.43	1.26	23.10
	Unthinned, nitrogen	66.85	1.94	23.25
	Thinned, nitrogen	70.98	1.57	24.10
	Thinned, no nitrogen	69.50	1.57	22.80
1933				
Feb. 27	Unthinned, no nitrogen	61.94	1.17	17.50
	Unthinned, nitrogen	64.18	1.36	19.85
	Thinned, nitrogen	69.97	1.27	21.07
	Thinned, no nitrogen	68.43	1.31	22.35
Apr. 15	Unthinned, no nitrogen	56.06	1.29	23.24
	Unthinned, nitrogen	51.63	1.21	18.40
	Thinned, nitrogen	57.33	1.38	18.00
	Thinned, no nitrogen	63.41	1.05	20.05
May 20	Unthinned, no nitrogen	62.40	.97	21.67
	Unthinned, nitrogen	48.36	1.29	16.98
	Thinned, nitrogen	51.50	1.34	17.06
	Thinned, no nitrogen	63.95	1.13	19.73
July 5	Unthinned, no nitrogen	66.73	.87	21.70
	Unthinned, nitrogen	45.71	1.14	15.66
	Thinned, nitrogen	48.20	1.25	15.22
	Thinned, no nitrogen	61.32	.93	16.97

[1] Planted Aug. 23, 1932.

Acid Hydrolyzable Polysaccharides

Seasonal changes in the percentage of acid hydrolyzable polysaccharides (2 percent hydrochloric acid) in the roots are, in general, similar to the trends shown by sucrose. The plants receiving nitrogenous fertilizer showed a steady decrease in complex carbohydrates during the period of seed production, while in the unfertilized plants the decrease in this fraction was much less marked. The variations in polysaccharides are of less magnitude than those found in sucrose but are sufficient to indicate that the general effect of the nitrogenous fertilizer was to cause a decrease in the more labile forms of carbohydrates of the roots, presumably owing to increased use of these substances in other parts of the plants.

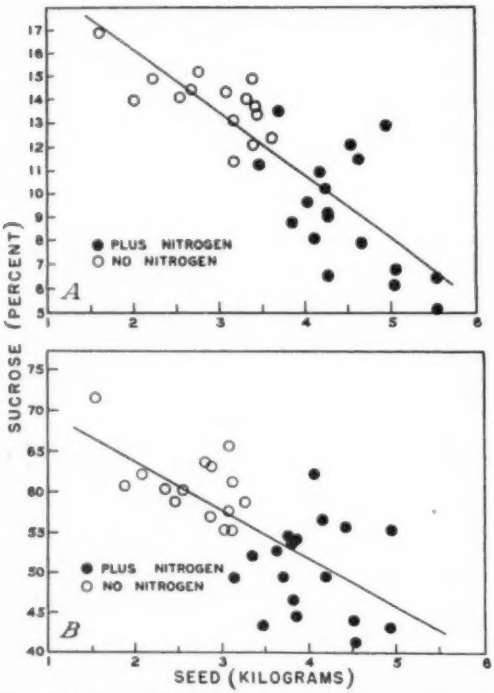

FIGURE 4.—Diagram showing relation between sucrose percentage on July 5, 1933, and seed yield of sugar beets. Results of the August 23 and September 15, 1932, plantings are combined in this graph: *A*, Fresh-weight basis (correlation coefficient, −0.830); *B*, dry-weight basis (correlation coefficient, −0.681).

Correlation between Sucrose in Roots at Harvest and Yield of Seed

When it became evident from the analyses of composite samples that nitrogen fertilization reduced the percentage of sucrose in the roots and at the same time increased the yield of seed, it was desired to obtain a more definite estimate of the relationship existing between these two factors. At harvest time, samples of roots were taken from 19 plots of the early planting and from 14 plots of the late planting. The percentage of sucrose in these samples was correlated with the yield of seed from the same plots. The relations between these two sets of observations are plotted in figure 4, with sucrose expressed both as percentage of fresh weight and as percentage of dry weight. The method of Wallace and Snedecor (*16*) was used in calculating the correlation coefficients. The results are summarized in table 8.

The highly significant negative coefficients obtained in every case where the sucrose percentage of the roots at harvest time was correlated with the yield of seed indicate that the sucrose removed from the roots during the period of seed production was utilized in increasing

the fruiting processes of the plants. The effect of nitrogen in furthering this utilization of sucrose is shown in figure 4 by the fact that the plots which received ammonium sulphate are definitely lower in sucrose and higher in seed yield than are the unfertilized plots.

When it is considered that samples from both thinned and unthinned plots, comprising widely different sizes of beets, were included when the estimates of correlation were made, it appears that the sucrose was utilized with about the same degree of efficiency whether it was contained in a single large root or a number of smaller roots.

TABLE 8.—*Correlations between percentage of sucrose in the roots at harvest time and yield of seed*

Correlation between—	Planting date	Value of r	Significant value (P=0.01)
	1932		
Sucrose (fresh weight) and seed yield [1]	Aug. 23	−0.823	0.575
	Sept. 15	−.757	.661
	Both dates	−.830	.449
Sucrose (dry weight) and seed yield [1]	Aug. 23	−.705	.575
	Sept. 15	−.676	.661
	Both dates	−.681	.449

[1] Determined July 5, 1933.

DISCUSSION

The results of these experiments show that from the time the beets were planted in August or September until the period of winter dormancy carbohydrates increased rapidly in the roots. The highest percentages of sucrose were found in root samples taken in December and January. Applications of nitrogenous fertilizers in the fall caused a gain in carbohydrate accumulation, probably as a result of the greater leaf surface available for photosynthesis.

During the same period of fall growth the percentages of nitrogenous compounds in the roots changed only slightly. Even where heavy applications of ammonium sulphate were made in the fall, there was no evidence that they caused any storage of nitrogen in excess of that which took place under conditions of average fertility. There was, however, some indication that the fertilizer caused an increase in the proportion of soluble nitrogen found in the roots in early spring, and it is possible that this condition rather than the percentage of total nitrogen is responsible for the earlier and more vigorous seedstalk production that is commonly observed where beets are given adequate supplies of nitrogen during the vegetative period.

After seedstalk development begins in the spring there occurs a rapid disappearance of carbohydrates and nitrogenous materials from the roots. The movement of these materials from the roots early in the season is undoubtedly related to their use in the development of new leaves and seedstalks. As soon as a sufficiently large leaf area has been developed, however, it would seem that the function of providing carbohydrates for the fabrication of leaves, flowers, and fruits might be taken over by the leaves, thus making unnecessary the further withdrawal of sugars from the roots. As shown in figure 1, *B*, such a condition was actually found in the unfertilized plants early in the flowering period. From the latter part of April until the seed was

harvested, the percentages of sucrose in the roots either decreased slightly or, as in the case of the unfertilized plants in 1933, showed a marked increase in sucrose during the last 2 months. As has been pointed out, this condition was correlated with a pronounced shortening of the flowering period, which in turn resulted in greatly decreased yields of seed.

The trend of the sucrose curves in plants that received a spring application of nitrogenous fertilizer presents a striking contrast. In these plants the percentages of sucrose in the roots decreased steadily from the time seedstalk development began until the seed was ready to harvest. This condition was associated with a lengthened period of flower production and was definitely correlated with higher seed yields. From the standpoint of securing maximum seed production, it seems clear that the plants must be so conditioned that some use is made of the carbohydrate reserves of the roots. This seems well shown as the functions of nitrogenous fertilizers are followed throughout these tests.

It may be pointed out that the economy of food reserves in the sugar beet differs somewhat from the situation encountered in other plants. Haller and Magness (*7*) conclude from studies with apples that by adjusting the size of the crop to provide the proper leaf area per fruit not only is the highest quality of fruit produced but also sufficient excess of carbohydrate is left over to allow for normal vegetative growth and the formation of fruit buds for the following season. In the case of forage crops and grasses (*6*, *8*), it has been found that frequent cutting accompanied by applications of nitrogenous fertilizers results in the depletion of carbohydrates stored in the roots, so that the plants are much less vigorous the following year. In these plants the development of top growth, whether it be fruits or vegetative structures, must be regulated to allow for the accumulation of reserves in the perennial organs. Since the parts of the sugar beet remaining after seed harvest are of little value, there is no advantage in conserving carbohydrates in the roots if they can be utilized in the production of a heavier seed crop. In the present study the carbohydrates in the roots did not approach exhaustion even with heavy nitrogen treatment, but the data presented indicate that maintaining soil nutrients, particularly nitrogen, at adequate levels throughout the season increases the use of these reserves in the production of seed.

SUMMARY

Tests conducted during two seasons have shown that nitrogenous fertilizers play an important role in the production of sugar-beet seed. Heavy applications of ammonium sulphate have resulted in marked increases in yield of seed, but no consistent differences in quality can be attributed to treatment.

Chemical analyses showed that carbohydrates and nitrogenous compounds attain maximum levels in the roots at the time of winter dormancy. After seedstalk development begins in the spring, sugars and nitrogen are withdrawn rapidly from the roots. The loss of stored nitrogen from the roots is only slightly affected by fertilizer treatment and continues until most of the nitrogen has been withdrawn.

The amount of sugar moved from the root appears to depend upon the supply of nitrogen in the soil. When nitrogen is continuously available during the fruiting period, the percentage of sucrose in the roots decreases steadily until the seed is matured. This condition is related to a long period of flower production and results in high seed yields. If nitrogen becomes a limiting factor during the fruiting period the loss of sucrose from the roots ceases and the accumulation of sucrose in the roots begins. Under these conditions the formation of flowers stops prematurely and seed yield from such plants is correspondingly low.

Highly significant negative correlations have been established between sucrose percentages found in the roots at time of seed harvest and seed yields. The utilization of stored reserves in the sugar-beet root has been found to be dependent upon the supply of nitrogen available to the plant during the fruiting period and, therefore, this study affords a better understanding of the physiological basis for a fertilization program.

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FAILURE OF DIAKINESIS AND METAPHASE PAIRING AND THE BEHAVIOR DURING MEIOSIS OF UNIVALENT CHROMOSOMES IN ZEA MAYS [1]

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INTRODUCTION

Variation and its causes are of decided importance to the plant breeder. Powers (*25*)[3] has obtained evidence to show that meiotic instability is associated with progeny variability of certain characters in *Triticum vulgare*. The investigations of Blakeslee, as reported by Davenport (*15*), Beadle (*1, 2*), and Bergner, Cartledge, and Blakeslee (*5*) prove that in certain cases meiotic instability is due to recessive genes, and therefore is definitely inherited. More information concerning the reactions of these genes and the interaction between these genes and the environment is desirable. The present paper deals with another case of failure of pairing during certain stages of meiosis and with the behavior of univalent chromosomes in *Zea mays* L.

MATERIALS AND METHODS

CYTOLOGICAL

The microsporocytes were killed in a mixture of 1 part glacial acetic acid to 3 parts absolute alcohol and later stored in 70-percent ethyl alcohol. The material involved in the studies on failure of diakinesis and metaphase pairing was the progeny of inbred lines of *Zea mays*, H93 and H94. Hayes [4] has furnished the following statement concerning the history of these inbred lines: "These descended from crosses where *Pr.* came from the parent in the 'prostrate culture' and the female parent was a linkage tester *wx* in H94 and *sh* in H93." The F_1 generations between H42 (22 somatic chromosomes) and H93 and H94 (20 somatic chromosomes) were backcrossed to the parents during the summer of 1931. The progeny of the backcrosses were grown in the summer of 1932, and progeny of the lines inbred by self-fertilization and the backcrosses were grown again in 1933. Belling's (*3*) iron-aceto-carmine method of staining the microsporocytes was used.

STATISTICAL

Unless otherwise stated, 50 meiocytes were examined in the diakinesis and metaphase I stages, 125 in the telophase I, and 125 in the metaphase II.

Since the proportion of bivalent to univalent chromosomes varied considerably between plants in the diakinesis and metaphase I stages, some method of determining the variability within plants was neces-

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[2] The writers are indebted to Dr. L. M. Humphrey for killing and fixing the material in 1931 and for making the crosses, and to J. V. Hastings for taking the photomicrographs shown in pls. 1 and 2.

[3] Reference is made by number (italic) to Literature Cited, p. 666.

[4] HAYES, H. K. Personal communication.

sary in order to ascertain whether the differences between plants could be accounted for by the errors of random sampling. As regards the proportion of bivalent to univalent chromosomes, the distribution of the 50 meiocytes studied for each plant might be expected to follow the binomial distribution. However, since the chromosomes occur in pairs (as homologues) and since the data are taken on the basis of the number of chromosomes occurring as bivalents rather than the number of bivalents, a theoretical variance two times greater than that of the binomial distribution would be expected. Fisher (*16, p. 72*) gives a method for testing whether the variability agrees with that of the binomial distribution. In table 1 the results are given for testing whether the variance of the data obtained from diakinesis and metaphase I is two times greater than that of the binomial distribution.

TABLE 1. *Test to determine whether the general trend of variability accords with the binomial distribution;* [1] *diakinesis and metaphase*

Plant no.	Diakinesis			Metaphase		
	$\sqrt{2X^2}$	Difference	Mean number of chromosomes as bivalents	$\sqrt{2X^2}$	Difference	Mean number of chromosomes as bivalents
H94–3R	13.75	−4.94	19.88	16.21	−2.48	19.80
H94–2R	17.60	−1.09	19.48	27.60	+9.00	18.96
H152–9	12.92	−5.77	19.64	13.35	−5.34	19.76
H152–2	18.52	−.17	16.32	19.14	+.45	13.20
H152–8	21.52	+2.83	15.92	25.49	+6.80	16.84
H152–1	20.46	+1.77	14.04	23.26	+4.57	14.92
H152–11	20.72	+2.03	5.64	21.92	+3.23	3.80
H152–3	16.76	−1.93	2.16	18.14	−.55	2.20
H93–1			20.00			20.00
H151–8	17.10	−1.59	18.44	15.06	−3.63	19.40
H151–12	17.53	−1.16	19.00	26.52	+7.83	18.60
H151–13	19.20	+.51	16.40	30.36	+11.67	14.44
H151–1	17.32	−1.37	7.48	24.28	+5.59	6.64

[1] $\sqrt{2(2n-1)}=13.93$ $\sqrt{3.6(2n-1)}=18.69$.

The trend of variability in diakinesis (table 1) is of particular interest. The differences given are those obtained by the formula $\sqrt{2X^2}-\sqrt{3.6(2n-1)}$. Owing to the extreme variability of this material, it is doubtful whether the variability of any plants other than nos. H94–3R and H152–9 are significantly different from 3.6 times that expected for the binomial distribution. The reduced variability of these is undoubtedly due to the large number of chromosomes per meiocyte occurring as bivalents, since values cannot exceed 20 (i. e., the total number of chromosomes).

From table 1 it can be seen that the general trend of variability in the metaphase stage does not accord with that of the binomial distribution, nor does $\sqrt{2X^2}$ approximate $\sqrt{3.6(2n-1)}$ as would be expected if the variability were 3.6 times that of the binomial distribution. The reason for this is the tendency of a few cells to exhibit extreme deviations. For example, in plant H94–2R, it was not uncommon to find an occasional meiocyte in which all the chromosomes were univalents, even though the average number of chromosomes occurring as bivalents during metaphase I was 18.96. The metaphase association is definitely more variable than that for diakinesis.

From these results it is evident that the theoretical variance for the diakinesis stage is 3.6 *pqn* and for the metaphase it would be somewhat larger. In making the comparisons between plants and stages the standard deviation was obtained by employing the formula $\sqrt{3.6\ pqn}$, and therefore in comparing stages the standard deviation would be somewhat small. The biological reasons, together with the statistical evidence supporting these reasons as to why the variance of the diakinesis stage is 3.6 *pqn*, are given in a paper by Powers and Clark.[5]

EXPERIMENTAL RESULTS

FAILURE OF PAIRING

The data on the proportion of bivalent chromosomes in the diakinesis and metaphase I stages of meiosis are given in table 2. These data have a direct bearing on two fundamental problems (1) the difference between stages in the same plant, as regards the proportion of bivalents to univalents, and (2) the difference between plants as represented by the microsporocytes examined. It is clear that in plants H152–2, H152–11, and H151–13 there is a statistically significant increase of univalents in the metaphase I stage. Also, the fact that the variance of the metaphase I stage is greater may be taken as convincing evidence that chromosomes in the bivalent condition at diakinesis may occur as univalents at metaphase I, because the extreme microsporocytes (in regard to the proportion of univalents to bivalents) previously noted must necessarily be in the direction of more univalents. Beadle (*1*) found that synapsis occurs, apparently normally, in asynaptic maize plants and that homologous chromosomes tend to disjoin during pachynema. The results of this study make it evident that homologous chromosomes also tend to separate between diakinesis and metaphase I.

TABLE 2.—*The ratio of chromosomes as bivalents to chromosomes as univalents in the diakinesis and metaphase I stage of meiosis.*

Plant no. and stage	Bivalent [1]	Univalent [1]	Difference	Weight of ears	Plant no. and stage	Bivalents [1]	Univalents [1]	Difference	Weight of ears
H94–3R:				*Grams*	H152–3:				*Grams*
Diakinesis	994	6			Diakinesis	108	892		106
Metaphase	990	10	4± 7.56		Metaphase	110	890	−2±26.44	
H94–2R:					H93–1:				
Diakinesis	974	26			Diakinesis	1,000	0		
Metaphase	948	52	26±16.39		Metaphase	1,000	0		
H152–9				64	H151–8				55
Diakinesis	982	18			Diakinesis	922	78		
Metaphase	988	12	−6±10.31		Metaphase	970	30	−48±19.07	
H152–2				88	H151–12				18
Diakinesis	816	184			Diakinesis	950	50		
Metaphase	660	340	156±36.72		Metaphase	930	70	20±20.13	
H152–8				77	H151–13				48
Diakinesis	796	204			Diakinesis	820	180		
Metaphase	842	158	−46±32.63		Metaphase	722	278	98±35.41	
H152–1				88	H151–1				38
Diakinesis	702	298			Diakinesis	374	626		
Metaphase	746	254	−44±37.88		Metaphase	332	668	42±40.51	
H152–11				60					
Diakinesis	282	718							
Metaphase	190	810	92±35.82						

[1] Number of individual chromosomes in the designated condition in 50 meiocytes.

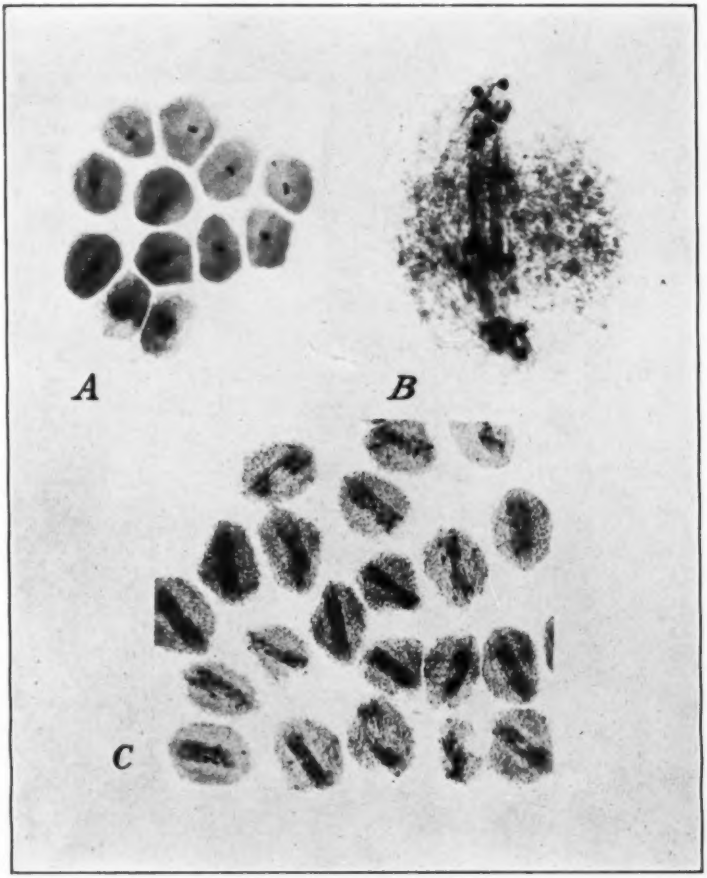
[5] POWERS, L., and CLARK, A. FAILURE OF CHROMOSOME PAIRING AS EVIDENCE OF SECONDARY DIPLOIDY IN ZEA MAYS. Unpublished manuscript.

In order to ascertain whether the differences between plants, as shown by the microsporocytes studied, exist as regards failure of pairing during diakinesis and metaphase, detailed statistical calculations are not necessary. Significant differences may be found in both lines H152 and H151. An examination of table 2 reveals that nearly all of the chromosomes occur as bivalents in the microsporocytes from plant H152–9, whereas the majority of the chromosomes occur as univalents in the microsporocytes from plant H152–3. The difference between these two plants is illustrated by plate, 1, *A* and *C*. Close observation of some of the cells of *C* will disclose bivalents on the equatorial plane, the members of which have not disjoined. Such scattering of the univalent chromosomes throughout the achromatic figure as can be noted in *C* and *B* is typical of those plants having a high proportion of univalents. The data together with the photomicrographs establish the fact that, in regard to the microsporocytes studied, differences between plants exist.

The question arises whether these differences are genetic or whether they are largely environmental. The data furnish some information on this point. Plants H94–3R and H94–2R from the inbred line giving rise to the inbred culture H152, and plant H93–1 from the inbred line giving rise to the inbred culture H151 do not show as high a percentage of univalents as either of the inbred cultures to which they give rise. The inbred cultures of H152 and H151 were grown a year later than their parental inbred lines, and it seems possible that the differences noted are mainly environmental. It is of interest that Hayes and Brewbaker (*17*) reported unexpected ratios of purple to red aleurone color in an F_2 generation involving these characters. Since the above inbred lines involve these characters likewise, and have been carried by the Minnesota Station for some time, it seems a plausible supposition that the abnormal ratios reported by Hayes and Brewbaker were due to failure of pairing during metaphase I. The most convincing evidence that the environment plays an important part in determining the proportion of bivalents to univalents comes from the data (table 2) which show that the ear weights are not associated with the degree of failure of pairing in the plants studied. Since Beadle (*1*) and Bergner, Cartledge, and Blakeslee (*5*) have shown that if their material sterility is high in plants having a high proportion of univalents at metaphase I, it would be expected that those plants in the present study having a high proportion of univalents would show a lower percentage of fertility than those with a low proportion of univalents, and in turn these differences in fertility should be reflected in ear weights. However, that they are not reflected in ear weights may be due to the fact that the behavior of univalents is such as to give a higher proportion of gametes carrying complete genoms than would be expected. The data bearing on this point are discussed in the following section.

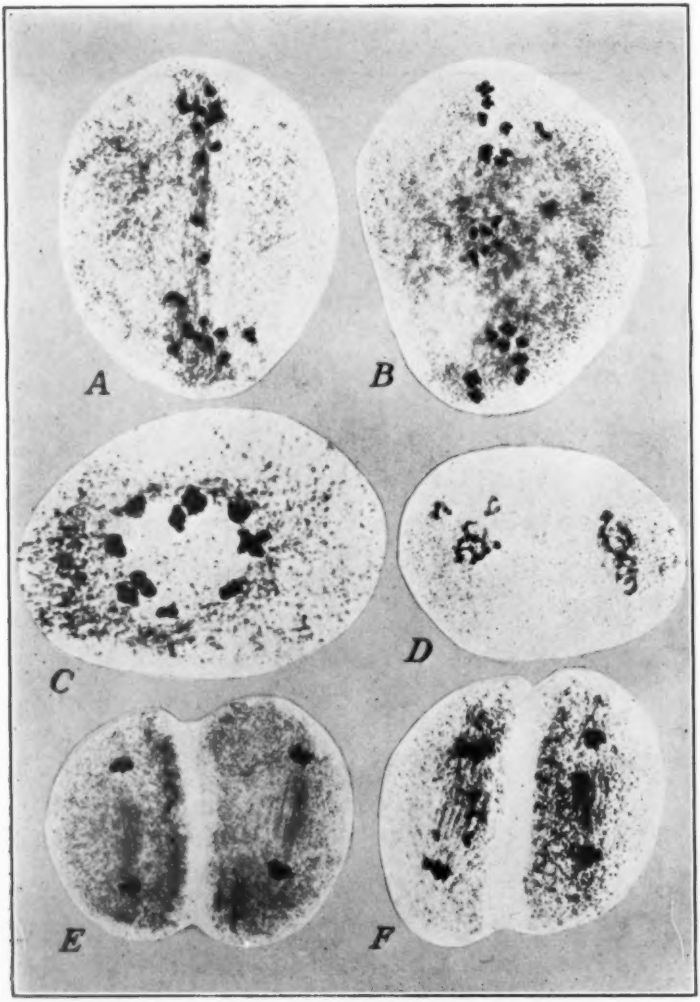
BEHAVIOR OF UNIVALENT CHROMOSOMES DURING CERTAIN STAGES OF MEIOSIS

To determine the frequency of the different distributions of chromosomes to the poles, counts were made of the number of chromosomes on either side of the equatorial plane of microsporocytes in early anaphase I. The determinations were made for plant H152–3. Plate 2, *A*, shows the stage at which the counts were made and plate



DEGREE OF FAILURE OF PAIRING.

For full description see text.



BEHAVIOR OF CHROMOSOMES DURING DIFFERENT STAGES OF MEIOSIS IN PLANTS SHOWING SOME DEGREE OF FAILURE OF PAIRING.

For full description see text.

2, *C*, shows 10 chromosomes in 1 member of the diad after division I. Examination of 35 cells gave the following results, 13 had a 10 by 10 distribution, 15 an 11 by 9 distribution, and 7 a 12 by 8 distribution.

Next, it is desirable to know whether the gametes arising from the meiocytes showing a 10 by 10 distribution possess a complete genom, which is necessary for gametic fertility. From observation and judging by size of chromosome, the impression was gained that homologues showed a decided tendency to be distributed to opposite poles. To determine whether such were the case the distribution in meiocytes showing only two univalents was studied. If there were no tendency for homologous chromosomes to be distributed to opposite poles, the chances would be 1 to 1 that the two univalents would be on the same side of the equatorial plane. On the other hand if the majority of microsporocytes show the univalent chromosomes to be on opposite sides of the equatorial plane, it can be concluded that there is a tendency among univalents for homologous chromosomes to travel to opposite poles. The results of the determinations are given in table 3. It is apparent that as far as these results are concerned the distribution is entirely random and the probability of the members of any one set of univalent and homologous chromosomes going to opposite poles is 0.5.

TABLE 3.—*Distribution of univalent chromosomes to the poles, in microsporocytes showing only two univalents*

Univalents on—	Cells for plant—			
	H152-2	H152-1	H152-8	H152-9
	Number	*Number*	*Number*	*Number*
Same side of equatorial plane	12	37	39	32
Opposite sides of equatorial plane	11	34	33	34

$\chi^2=0.545$. P lies between 0.95 and 0.90.

Chromatin loss would play a part in causing sterility. The formation in telophase I of both the usual nucleus and the so-called "micronucleus", which might be better called mikrokaryon,[5] is seen in plate 2, *D*. Plate 2, *F*, is a photomicrograph of a cell in early telophase II which shows lagging chromosomes. Plate 2, *B*, shows three univalent chromosomes undergoing the equational division in division I. It is well to note that the upper and lower poles of this cell possess nine and eight chromosomes respectively. Powers (*25*) has shown that in *Triticum* this behavior results in chromatin loss in division II. Examinations were made of 125 meiocytes in telophase I and 125 cells in telophase II, in order to determine the frequency of chromosome loss. The number of microsporocytes showing chromatin loss is listed in table 4. The following conclusions may be reached by an examination of table 2 and by a comparison of tables 2 and 4. There is a positive association between the degree of failure of pairing during diakinesis and metaphase I and chromatin loss, and there is a close relationship between chromatin loss in divisions I and II.

[5] From mikros, small, and karyon, nucleus. While the term "micronucleus" has been used for extra nuclear chromatin fragments existing after telophase, it seems preferable to use the new term "mikrokaryon." According to Newman (*24*) "micronucleus" was proposed, and is properly used, for the smaller of the two nuclei in *Paramecium* and its allies.

TABLE 4.—*Relationship between the number of microsporocytes showing lagging chromosomes in the telophase of division I and the number showing chromatin loss in telophase II*

Telophase	Cells for plant—			
	H152-11	H152-2	H152-8	H152-3
	Number	*Number*	*Number*	*Number*
I	29	18	14	111
II	28	14	12	78

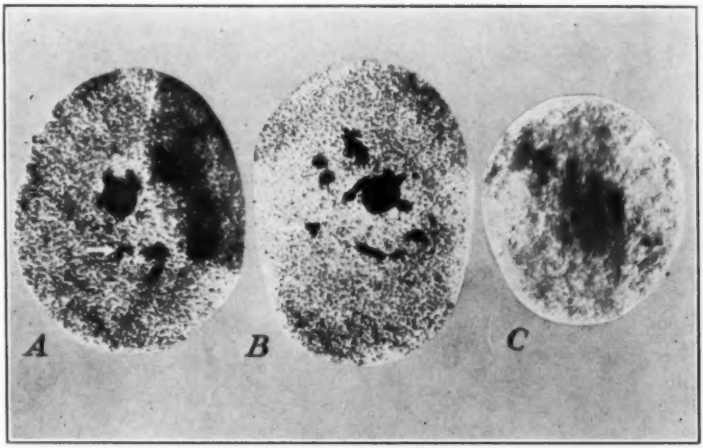
$\chi^2=1.191$. P lies between 0.80 and 0.70.

From these results it may be concluded that the behavior of the univalent chromosomes during meiosis is such that in plants showing a fairly high proportion of failure of conjugation during diakinesis and metaphase, nonfruitfulness would be expected to be high. That such was not the case may be due either (1) to the fact that the proportion of bivalent to univalent chromosomes was not the same for the megasporocytes and microsporocytes, or (2) to environmental influences, such perhaps as daily fluctuations in temperature, and therefore the behavior of the microsporocytes studied may not have been typical of the sporogenesis of the gametes giving rise to the kernels on the self-fertilized ears of corn. From the work of Beadle (*1*) showing that sterility is high in those plants having a high porportion of univalents at metaphase I, the latter explanation seems the more plausible. Belling (*4*) and Sax (*33*) have noted the disturbing effects of temperature upon meiosis.

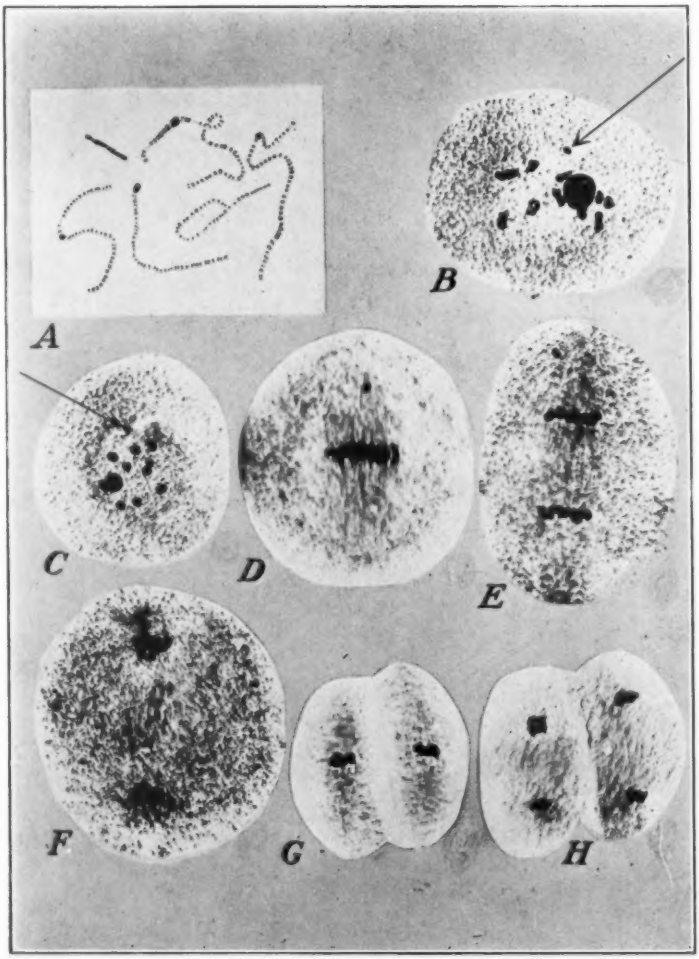
BEHAVIOR OF AN EXTRA CHROMOSOME DURING MEIOSIS

Randolph (27) reports extra chromosomes in *zea mays*, and he (*28*) has designated the normal monoploid set of 10 chromosomes as the A type and the supernumerary chromosomes commonly found in Black Mexican sweet corn as the B type. The extra chromosome reported in this study cannot be identified as belonging to the normal monoploid set of 10. The history of this particular extra chromosome has been given by Humphrey (*19*), together with the behavior of the 22-chromosome parent entering into the crosses reported in this study.

The behavior of the extra chromosome was determined by a study of microsporgenesis in two F_1 plants derived from crossing the 22-chromosome line with a 20-chromosome line inbred by self-fertilization. The results of the study are illustred by plate 3. *A* is a camera-lucida drawing of 6 of the 11 chromosomes in the pachytene stage of prophase I. From the diagram of the 10 haploid chromosomes given by Rhoades and McClintock (*31*) it would seem that the short chromosome without a trabant and nearest the center is undoubtedly No. 10 of McClintock's diagram (*22*). McClintock found the B type of chromosome slightly more than one-half the length of the shortest member of the normal complement. From Plate 3, *A*, it can be seen that the extra chromosome (upper left-hand corner) is not quite half the length of No. 10. It is denser than the other chromosomes and appears to have pyknotic regions somewhat similar to those of the B type chromosomes described by McClintock (*21*).



BEHAVIOR OF AN EXTRA UNIVALENT CHROMOSOME DURING MEIOSIS.
For full description see text.



BEHAVIOR OF TRISOMIC EXTRA CHROMOSOMES DURING DIAKINESIS AND METAPHASE I.

For full description see text.

The arrows in plate 3 *B* and *C*, show the extra chromosome in two different stages of diakinesis. In this stage of the prophase the extra univalent chromosome can be distinguished from the 10 normal bivalents by its size and by the fact that in the great majority of cases it assumes a position on the periphery of the karyolymph near the disappearing nuclear membrane. Statistical determinations on chromosome behavior were made. In the diakinesis stage (pl. 3, *B* and *C*), 202 microsporocytes were examined, all of which showed 10 bivalent and 1 univalent chromosome. Of 214 cells examined in metaphase I, all but 7 showed the single chromosome proceeding toward one pole (pl. 3, *D*). A total of 114 anaphases were examined and in only 4 of these were lagging chromosomes observed. The majority of the remaining cells showed the extra chromosome near the pole as in plate 3, *E*.

Plate 3, *F*, illustrates the early telophase in which the extra chromosome is being included in the upper nucleus. Of a total of 148 cells examined in the metaphase stage of division II, only 7 possessed chromosomes not oriented upon the equatorial plane. The regularity of metaphase II can be seen from plate 3, *G*. Sixty-nine meiocytes were examined in early telophase II (pl. 3, *H*). None of these showed lagging chromosomes. One thousand immature microspores in the quartet stage were examined, and in only seven was a mikrokaryon found in addition to the normal nucleus. For comparative purposes, meiosis was studied in 20- and 22-chromosome plants. A statistical summary of their meiotic behavior is given in table 5.

TABLE 5.—*Meiotic behavior in 20- and 22-chromosome plants*

20-CHROMOSOME PLANT

Meiotic stage	Cells examined	Cel's showing—				
		10_{II}	$9_{II}+2_I$[1]	Lagging chromosomes	Nonorientation	Mikrokaryons
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
Diakinesis	100	96	4			
Metaphase I	142	131	11			
Telophase I	221			10		
Metaphase II	44				4	
Quartet	1,000					9

22-CHROMOSOME PLANT

Meiotic stage	Cells examined	Cells showing—					
		11_{II}	$9_{II}+1_I+1_{III}$	$10_{II}+2_I$	Lagging chromosomes	Nonorientation	Mikrokaryons
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Diakinesis	100	100					
Metaphase I	69	67	1	1			
Telophase I	100				3		
Metaphase II	100					8	
Quartet	500						1

[1] $9_{II}+2_I$ signifies 9 bivalents plus 2 univalents.

From an examination of these data it is apparent that chromatin loss is no greater in the 21-chromosome plant than in either the 22- or the 20-chromosome plants. This extra chromosome in the univalent condition passes to one or the other pole during division I, and its behavior during the remainder of meiosis is the same as that of any other chromosome. Trivalent associations with any other chromosome are rarely if ever formed. Since this extra chromosome could have arisen by fragmentation from one of the A type chromosomes, the question arises as to whether it can form multivalent associations. A sister plant to the 22-chromosome plant used as a parent in these studies was found to have 23 chromosomes. Meiosis in this plant should furnish additional information concerning the extra chromosome under consideration.

The behavior during certain stages of meiosis of the trisomic extra chromosome is illustrated in plate 4. A total of 154 cells in the diakinesis stage was examined; of these 91 showed 11 pairs and 1 single and 63 showed 10 pairs and 1 trivalent. Two types of trivalent association commonly found are shown in plate 4, *A* and *B*. In metaphase I, 271 cells were examined, 146 of which showed 11 bivalents and 1 single; 114 showed 10 bivalents and 1 trivalent; 6 showed 2 singles, and 5 three singles. Precocious trivalent chromosomes are shown in plate 4, *C*. The number of chromosomes approaching each pole was determined for 15 meiocytes in the anaphase I stage, and all showed 11 chromosomes approaching one pole and 12 chromosomes approaching the other. In those cases in which one of the extra chromosomes was univalent, it usually preceded the other chromosomes. An examination of the 82 meiocytes in late telophase I showed that chromosome loss, if occurring, was rare, since no chromatin was found outside of the partially reconstructed nuclei. Only 3 of the 500 microsporocytes in the quartet stage showed chromatin loss.

The studies with the extra chromosome trisomic provide the following information: According to Darlington's (*13*) theory that the chromosomes in the later prophase and metaphase are kept from disjoining by chiasmata, the trivalent associations would mean that these extra chromosomes were of sufficient length to form at least two chiasmata. Then the lack of multivalent associations from pachynema to metaphase I, inclusive, could not be due to the inability of the extra chromosome to form multivalent associations in the 21-chromosome plants studied.

Studies of this chromosome in prophase I (pachynema) of 21-chromosome plants show that, in general, it is made up of four to seven pairs of pyknotic areas. These pyknotic areas are not paired in the sense of the bivalent chromomeres seen in the rest of the nuclear complex but are split chromomeres. The univalent is then made up of two chromatids (sister chromatids) as contrasted with the two homologous chromosomes which constitute a bivalent. Thus, at either late zygonema or early pachynema, the univalent would be prematurely in the very late diplotene condition. Humphrey's studies (*20*) on extra chromosomes in Black Mexican sweet corn have shown that at middle diplonema the univalent has already contracted until it is essentially equivalent to the condition seen at late diakinesis. The precocious nature of the univalent appears to

be further emphasized by its texture and staining reaction. In general, there is an increase in chromaticity of chromosomes as they approach the metaphase condition, those at metaphase being more deeply stained than at pachynema. Even in the earliest prophase (zygotene) cells in which the arrangement of chromosomes was such as to reveal the univalent, it was noticeably deeper in color and smoother in texture than were the bivalents, which is in agreement with the findings of Randolph (*28*). Possibly, the evidently precocious nature of this univalent accounts, in part, for its general independence with respect to the chromosomes of the normal complement.

BEHAVIOR OF THE EXTRA CHROMOSOME AS DETERMINED BY INHERITANCE STUDIES

From the meiotic behavior of the extra chromosome, 50 percent of the gametes of the 21-chromosome F_1 plants would be expected to possess this chromosome. To test whether such were the case, the F_1 plants were backcrossed to progeny of both the 20- and 22-chromosome parents, and the chromosome number of the resulting plants was determined.

The material could be divided into two parts—that in which the F_1 was used as the female parent and that in which the 21-chromosome plant was used as the male parent. When the F_1 was used as the female, the ratio of plants carrying the extra chromosome to those not carrying it was 24:26; when the F_1 plant was used as the male parent the ratio was 15:34. These two sets of data would be expected to follow the binominal distribution, and therefore by applying the standard deviation for the binominal distribution it can be determined whether the deviations from a 1:1 ratio can be accounted for by errors due to random sampling. Statistical analysis is not necessary to ascertain that the fit between the obtained and the expected is good when the 21-chromosome F_1 plant was used as the female parent. The standard deviation for the test in which the F_1 was used as the male parent was 3.5, which means that the odds against the deviations noted being due to the errors of random sampling are greater than 99:1.

In drawing conclusions from these data the researches of other investigators are interesting. In triploid maize, McClintock (*21*) noted a decided selection against male gametes carrying extra chromosomes and a less obvious selection against eggs carrying extra chromosomes. This is also in accord with data from studies on trisomic maize (*23*). These findings are in agreement with those of Buckholtz and Blakeslee (*7, 8, 9, 10, 11*), Davenport (*14*), Blakeslee and Cartledge (*6*), Randolph (*30*), and others. The fact that when the F_1 with 21 chromosomes was used as the male parent, the microspores with 10 normal chromosomes plus 1 extra chromosome did not bring about fertilization as often as normal microspores (i. e. those with 10 chromosomes) shows that the behavior of this extra chromosome is similar to that of duplicated chromatin material. The fact that microspores carrying the extra chromosome do not bring about fertilization as frequently as those not possessing the extra chromosome makes it apparent that this chromosome is not composed entirely of inert chromatin but must carry at least one gene. One other finding should be mentioned, namely, that a progeny test showed some tendency for the extra chromosomes to increase.

DISCUSSION AND CONCLUSIONS

Variability is of extreme importance in any crop improvement program. By inducing variability selection of the desired type is made possible. After the desired type has been selected, it is highly important to maintain that type. One of the greatest obstacles to maintenance of type is natural crossing. Another cause of variation and one which probably plays an important part is meiotic instability.

The causes and consequences of meiotic instability are important because an understanding of them may lead to the formation of methods designed to take this character of plant material into consideration.

Turning to the effect of environment, the work of Stow (*34*, *35*) merits consideration. He found that meiosis in the potato is especially sensitive to high temperatures. In his material conjugation took place normally at 20° C.; whereas, in rising temperatures the power of conjugation decreased and was altogether suspended at 25° to 30°. Heilborn (*18*), investigating the effects of temperature on meiosis in apples, found that a slight rise in temperature prevents conjugation in some varieties. Among certain varieties temperatures as low as 20° were injurious, whereas other varieties withstood temperatures as high as 35°. Bergner et al. (*4*) believe that in homozygous *bd* daturas, the variability in the distribution of chromosomes at anaphase I and in the amount of nonreduction and the variability in the amount of pollen fertility are probably the results mainly of environmental factors. Randolph (*29*) has noted that heat treatment resulted in abnormal synaptic behavior of chromosomes during meiosis. In the present study it seems probable that environmental factors had a decided effect upon the degree of failure of pairing at diakinesis and metaphase I.

The work of Blakeslee (*15*), Beadle (*1*, *2*), Sapêhin (*32*), and Bergner, Cartledge, and Blakeslee (*5*) definitely proves that there are genes which affect meiosis, the dominant alleles of which produce stability, whereas their recessive alleles are conducive to instability. Such genes have been found for *Datura*, *Zea*, and *Triticum*. It seems highly probable that the failure of pairing during metaphase I reported by Clausen (*12*) for *Nicotiana* and Yamamoto (*36*) for *Rumex* is subject to genic control.

From the data available, it can be concluded that meiotic instability is genetic in some cases at least and that this character is definitely susceptible to environmental influences.

Some information is available concerning the consequences of meiotic instability and deserves consideration here. It is common knowledge among agronomists and plant breeders that some varieties of wheat during certain years are notoriously variable. Supreme wheat, a variety of *Triticum vulgare*, is of this type. This variability has usually been attributed to natural crossing. However, speltoid types appearing among the progeny of Supreme are not readily explained by natural crossing. On the other hand, cytological investigations (data not published) have shown that chromatin loss in Supreme was high and that 41-chromosome plants were of frequent occurrence during the season that this variety was studied. Powers (*25*, *26*) has shown that in at least one of the varieties studied irregular meiotic behavior such as chromatin loss, nonorientation, and failure

of pairing during metaphase is associated with variability of characters of the progeny of plants showing these irregularities of meiosis.

The information available at present is sufficient to warrant more attention being given to the character meiotic instability. Also, it is apparent, since this character is so readily influenced by the environment, that the experimental design for studying meiotic instability will have to be as thoroughly worked out as that for studying any quantitative character, as, for example, yield. Further research is necessary to determine the number of years a selfed line or strain will have to be investigated to determine its degree of meiotic stability. The data now available make it seem likely that certain selections may appear in which meiosis may seem regular, and then when other environmental conditions are encountered cytological studies will reveal meiotic instability. The experimental design must provide for an adequate sample to ascertain the variability within plants, between plants, between strains or selections, and between environmental conditions, particularly those encountered between years.

The results obtained from a study of the extra chromosome deserve some consideration, as the behavior of the extra chromosome differs somewhat from that found by Humphrey (*20*) for the supernumerary chromosomes of Black Mexican sweet corn. In both his material and that studied by the writers there was found a tendency for the extra chromosomes to increase. However, the results differed in that Humphrey found irregular meiotic behavior which would account for the increased chromosome number in the resulting progeny. After reviewing his article the writers reexamined their material with the results reported above, namely, that irregularities were no greater in the 21- and 22-chromosome plants than in the 20-chromosome plants. Even in the 21-chromosome plant chromatin loss was no more frequent than in the 20-chromosome plants, for the extra chromosome arrived at one or the other pole in division I and behaved like any other chromosome in division II. The behavior of the extra chromosomes during meiosis in the 22-chromosome plant was regular, which agrees with the findings of Humphrey (*19*), who studied the parental lines of the material reported herein. Some nonorientation was found, but the frequency of this irregularity was just as high in material not carrying any of the extra chromosomes. However, it could be possible that nonorientation resulting in nondisjunction of the extra chromosome would result in a considerably higher proportion of functional gametes and viable zygotes than if the same phenomenon occurred with the A-type chromosomes.

Another finding in addition to those in previous reports (Rhoades and McClintock (*31*) and Humphrey (*20*)) is that the extra chromosome is carried more frequently through the megaspores than through the microspores. In this respect the extra chromosome reported in this study behaves like duplicated chromatin material and, as has been shown, must carry at least one gene. Since the behavior of the extra chromosome reported herein differs in many respects from that of the B-type chromosomes studied by previous workers, it is believed that a different type of chromosome may be involved. It should be pointed out that the findings of the writers are in accord with those of Humphrey (*19*), who reported on the 22-chromosome inbred lines used in this study.

SUMMARY

The results of a study on the failure of diakinesis and metaphase pairing and the behavior during meiosis of univalent chrososomes in *Zea mays* are reported.

The variance of the data taken on the proportion of bivalents to univalents for diakinesis was found to be 3.6 times that of the binomial distribution. It seems that in studies such as this the variance of the species under consideration should be determined in order to obtain a reliable estimate of the standard deviation.

It was found for meiocytes possessing only two univalents that the distribution of chromosomes during anaphase I was entirely random for univalent chromosomes, and hence the probability of the members of any one set of univalent and homologous chromosomes going to opposite poles was 0.5.

A positive association was found between the degree of failure of pairing during diakinesis and metaphase I and chromatin loss.

The data indicate that the degree of failure of pairing during diakinesis and metaphase I is affected by the environment.

The behavior of the extra univalent chromosome during meiosis was unique as compared to the normal complement in that it always preceded the members of the bivalent complements to the poles and was not lost in either division I or II. This conclusion was reached through a study of meiosis and was substantiated by the inheritance studies in which the extra chromosome was carried by the megasporocytes.

A reduction in the proportion of plants possessing the extra chromosome when the 21-chromosome F_1 individual was used as the male parent compared to the increased proportion of plants possessing the extra chromosome when the F_1 was used as the female parent shows that the microspores possessing the extra chromosome do not bring about fertilization as frequently as those not possessing it. Then it is evident that this extra chromosome is not completely composed of inert material, but must carry at least one gene.

Attention is called to the prevalence of meitoic instability and therefore the importance of considering it in breeding programs and the necessity for further research to determine the proper experimental design for studying this character of plant material.

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CHEMICAL COMPOSITION OF AVOCADO FRUITS[1]

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INTRODUCTION

In a previous paper by the writer (*6*)[2] it was pointed out that the distribution of the stomata in fruits of avocado (*Persea gratissima* Gaertn.) is governed by growth and is related to the water loss and aeration of the fruit. As a result of this work, the number of stomata, the water loss, and the permeability of avocado fruits were shown to be greater in the tip than in the stem halves. The nonuniformity of these factors in the various portions of the fruit skin suggested the need for a study of the chemical composition of the various portions of avocado fruits.

Data regarding the composition of avocado fruits are limited primarily to the values for the fat and sugar content, for it is these constituents that determine in a large measure the quality of the fruits. Little is known regarding the iron, copper, and manganese content, although these elements are of considerable importance in plant and animal nutrition.

Avocado trees frequently are grown in districts in which the soil or irrigation water or both contain large concentrations of salts, chiefly chlorides or sulphates. In such locations the leaves may be seriously tip-burned. So far as the author is aware, no data exist from which it is possible to learn the extent of the accumulation of chloride, sulphate, and other constituents in the fruits. This paper presents data regarding not only the occurrence and distribution of certain constituents in avocado fruits but also some of the changes that accompany increasing maturity.

PREVIOUS WORK

The composition of a single sample of ash in avocado fruit is reported by Jaffa and Goss (*9*). Church and Chace (*1*) found that a marked increase in the fat content during the rapid development of the fruits was accompanied by a decrease in total sugar.

Attention is called by Church and Chace (*1*) to an analysis in which they found the outer half of the fresh pulp next to the skin to contain 1.54 percent of ash as compared with 1.36 percent in the inner half next to the seed. Differences of this kind in other fruits have recently been referred to by Haas and Klotz (*3*), Drain (*3*), and Tucker (*12*), and in the vegetative portions of plants by Mason and Maskell (*10*), Frear (*4*), Tukey and Green (*13*), and Cowell (*2*). Such investigations have shown the nonhomogeneous constitution of the tissues that make up certain organs, whether these be vegetative or reproductive in nature.

[1] Received for publication Aug. 24, 1936; issued June 1937. Paper no. 359, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, Calif.

[2] Reference is made by number (italic) to Literature Cited, p. 686.

METHODS OF ANALYSIS

Unless otherwise stated, the fruits[3] after being picked from the trees, were wiped free of dust, washed with distilled water and dried with filter paper, and then weighed. In some cases the pulp of the fruits was cut into halves from stem end to tip end, in other cases these halves were further subdivided into inner and outer portions. The seed and skin were removed and weighed, care being taken to free the skin from adhering pulp.

Finely cut samples of fresh material (100 g) were used for the determination of inorganic constituents and were dried below 65° C. in a current of air in a large oven. Samples of fresh material (25 to 50 g) for the determination of total and reducing sugars consisted of thin segments which were finely cut and weighed prior to being dropped into enough 95-percent boiling alcohol to secure a strength of 80 percent. The boiling of the alcohol was continued for a short time in order to kill the tissue. The Erlenmeyer flasks (300 cc) were then tightly closed with stoppers protected with tinfoil. When cool, the alcohol was measured and run into a 500-cc volumetric flask used for sugar extraction. The avocado material was finely macerated in a mortar with coarsely pulverized quartz and was transferred to the 500-cc flask with the aid of 80-percent alcohol. The alcohol was then reduced to 50 percent strength and the total and reducing sugars were determined by the method used by Haas and Klotz (*8*).

The fat determinations were made on samples of fresh material (0.5 to 1.0 g) by the use of anhydrous ether extractions from thimbles containing anhydrous copper sulphate, sand, and asbestos layers. The other chemical analyses for the most part were conducted by the methods employed with date fruits by Haas and Bliss (*7*). In many cases sulphuric acid was added to the dry matter before ashing (muffle furnace at low heat combined with leaching of the ash) in the determination of potassium. No appreciable losses of potassium were found when the acid treatment was omitted.

ANALYSES

DRY MATTER IN PULP

The results for freshly picked fruits (table 1) show somewhat more dry matter in the tip than in the stem portions of the pulp (without skin). Earlier determinations were not so uniform, largely because the fruits were not obtained directly from the trees.

The tip halves of avocado leaves (table 2) from which the midribs have been removed, contain greater percentages of dry matter in the fresh weight than do the stem halves. In this regard the leaves and fruits resemble one another, and it is of interest to note that fruits originate from megasporophylls, which are modified leaves.

ACIDITY OF PULP

No reference to the reaction of avocado pulp has been found in the literature.

[3] All fruit samples, unless otherwise specified, were collected at the Citrus Experiment Station.

TABLE 1.—*Dry matter in pulp (without skin) from different portions of avocado fruits of several varieties collected on different dates, 1930*

Variety and date of collection	Fruits	Dry matter on fresh-weight basis in—								
		Stem halves	Tip halves	Stem-end thirds	Middle thirds	Tip-end thirds	Stem-end quarters	Second quarter from stem ends	Third quarter from stem ends	Tip-end quarters
Blake:	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Aug. 21	16	20.74	21.34							
Aug. 25	15	22.70	22.64							
Aug. 28	5	22.35	22.99							
Benik:										
Aug. 21	4	11.73	12.37							
Aug. 25	10	12.90	13.78							
Puebla:										
Aug. 21	17	12.03	12.81							
Aug. 25	15	14.10	14.67							
Aug. 28	5	12.48	13.77							
Fuerte:										
Aug. 21	10	13.24	14.12							
Aug. 25	4	13.82	14.37							
Do	10	14.98	15.57							
Blake:										
Aug. 21	8			20.61	20.50	21.43				
Do	8			21.10	21.16	22.19				
Puebla seedling:										
Sept. 3	5			19.06	19.54	21.32				
Sept. 5	8			19.67	21.09	22.30				
Sharpless(?) seedling:[1]										
Nov. 22	1						25.49	27.12	27.27	28.47
Do	1						25.84	25.52	26.34	28.16

[1] Fruits 7 cm largest diameter by 19 cm long.

TABLE 2.—*Dry matter in tip and stem halves from four varieties of mature avocado leaves, with midribs removed*

Variety	Leaves	Dry matter on fresh-weight basis	
		Tip halves	Stem halves
	Number	*Percent*	*Percent*
Blake	180	37.19	35.35
Fuerte	194	38.94	37.89
Benik	189	33.72	32.42
Puebla	194	35.48	34.38

In June 1934 several mature avocado fruits were picked from a Guatemalan seedling tree and allowed to soften. One of the fruits, when edible, was cut into stem and tip halves. The seed was discarded and the skin removed. The pulp of the stem and tip halves was divided into inner and outer portions. The four samples of pulp were placed in beakers, and a minimum of distilled water was added to permit the maceration of the pulp into a paste. The pH values (table 3) were then determined by means of the quinhydrone electrode.

The actual acidity increases outwardly toward the skin and also increases from the stem toward the tip, in the inner as well as in the outer portions.

The pulp from another fruit of the same lot which was just beginning to soften but was not edible as yet, was cut into very small pieces and then macerated in a mortar with the aid of coarsely pulverized quartz. The pH values recorded in table 3 confirm the results obtained in the first experiment.

TABLE 3.—*pH values in different portions of mature avocado pulp (without skin) before and after it became edible, fruit from Guatemalan seedling being used*

Portion of fruit used	Soft and edible pulp	Hard, inedible pulp
	pH	*pH*
Stem half, inner portion	6.86	6.56
Stem half, outer portion	6.72	6.46
Tip half, inner portion	6.64	6.52
Tip half, outer portion	6.44	6.34

The pulp of avocado fruits is more acid near the skin. This indicates that the outer tissues are better aerated and that the carbon dioxide which tends to make the tissue more alkaline (*11*) is more quickly removed. The greater number of stomata in the tip half (*6*) probably also permits greater oxidation than in the stem half.

FAT CONTENT

Samples of avocado fruits were tested for fat content immediately after they were picked. Long, thin slices of pulp (without skin or seed) were cut in a direction extending from the stem or tip ends to the central crosscut, and these slices, in turn, were cut into narrow pieces. After the samples were weighed, the fat was extracted as described under Methods of Analysis.

The percentage of fat found in the fresh pulp of avocado fruits of different varieties picked at various stages of development are given in table 4. The fat content increased with increasing maturity of the fruits, as was found by Church and Chace (*1*). The fat content of the stem and tip halves showed no uniform differences.

TABLE 4.—*Fat content of the pulp (without skin) from the stem and tip halves of avocado fruits of several varieties in relation to time of collection*

Variety and date of collection	Fruits	Fat on fresh-weight basis		Variety and date of collection	Fruits	Fat on fresh-weight basis	
		Stem half	Tip half			Stem half	Tip half
Fuerte:	*Number*	*Percent*	*Percent*	Benik—Continued.	*Number*	*Percent*	*Percent*
Sept. 23, 1930	7	5.40	5.67	Feb. 16, 1931	8	11.35	11.47
Oct. 23, 1930	2	8.77	7.85	Apr. 14, 1931	5	19.00	20.23
Nov. 26, 1930	8	13.66	13.80	May 29, 1931	4	24.21	23.62
Dec. 29, 1930	10	15.72	14.12	Puebla seedling:			
Feb. 10, 1931	10	20.81	20.17	Oct. 10, 1930	10	15.34	16.68
Apr. 13, 1931	10	28.87	28.32	Nov. 21, 1930	4	21.79	29.54
Blake:				Puebla:			
Sept. 15, 1930	6	14.24	11.56	Oct. 23, 1930	10	6.94	5.68
Oct. 10, 1930	10	15.35	15.08	Nov. 21, 1930	10	9.75	8.26
Benik:				Jan. 6, 1931	12	14.24	13.59
Sept. 23, 1930	7	1.73	1.78	Feb. 4, 1931	10	15.20	17.80
Nov. 26, 1930	8	7.47	7.67				
Jan. 6, 1931	9	10.19	9.54				

REDUCING AND TOTAL SUGARS

Church and Chace (*1*) showed that while the total sugar content of the pulp of avocado fruits decreased, the fat content increased. In the present experiments, the fruits were utilized immediately after they were picked in order to avoid changes that are known to affect

avocado fruits in storage. Determinations (table 5) were therefore made of the reducing as well as the total (as reducing) sugar content of the stem and tip halves of the pulp (without skin) of several varieties of avocado fruits.

TABLE 5.—*Sugar content of the pulp (without skin) from the stem and tip halves of avocado fruits of several varieties in relation to time of collection*

Variety and date of collection	Fruits	Reducing sugars on basis of—				Total sugar as reducing sugar on basis of—			
		Fresh weight		Dry matter		Fresh weight		Dry matter	
		Stem half	Tip half	Stem half	Tip half	Stem half	Tip half	Stem half	Tip half
Blake:	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Oct. 10, 1930	10	0.54	0.34	2.09	1.40	0.65	0.52	2.57	2.15
Puebla seedling:									
Oct. 10, 1930	10	.73	.45	2.81	1.52	.90	.58	3.47	1.97
Nov. 21, 1930 [1]	4	.36	.23	1.03	.54	.64	.71	1.85	1.66
Puebla:									
Nov. 21, 1930	10	1.27	.80	6.71	4.16	1.65	1.34	8.71	6.99
Jan. 6, 1931	12	1.12	.77	4.68	3.13	1.46	1.32	6.19	5.39
Feb. 4, 1931	10	1.17	.76	4.33	2.58	2.58	1.03	9.53	3.53
Benik:									
Jan. 6, 1931	9	1.48	.84	7.05	3.62	1.89	1.06	8.98	4.60
Feb. 16, 1931	8	1.09	.80	4.90	3.29	1.27	1.06	5.72	4.52
Apr. 14, 1931	5	.70	.49	2.34	1.56	.87	.74	2.93	2.38
May 29, 1931	4	.25	.19	.80	.60	.49	.47	1.57	1.45
Fuerte:									
Feb. 10, 1931	10	.59	.29	1.98	.98	2.05	1.74	6.84	5.87
Apr. 13, 1931	10	.40	.19	1.11	.51	.47	.55	1.30	1.51
Apr. 30, 1931 [2]	6	.13	.14	.36	.48	.31	.25	.99	.86

[1] Last of crop. [2] Overmature.

The pulp of the stem halves of avocado fruits usually contains more reducing and total (as reducing) sugars than that of the tip halves, and in many cases the nonreducing sugar is higher in the tip than in the stem halves. The differences in sugar content of the halves decreased as the fruits became fully ripe or overmature on the tree. The data confirm the results of Church and Chace (*1*) in indicating a reduction in sugar content with increasing maturity of the fruits. In date fruits of the Deglet Noor variety (*7*), and in citrus fruits (*8*), higher percentages of reducing sugars have been found in the tip than in the stem portion.

INORGANIC CONSTITUENTS OF THE FRUIT

Prior to 1922 when Church and Chace (*1*) incidentally referred to an increase of 0.18 percent in the ash content of the outer, over that of the inner portion of the pulp of an avocado fruit, it was assumed that the skin and pulp were each in themselves uniform in inorganic composition. The results of the present investigation indicate that there is no uniform distribution of the various constituents in avocado fruits.

The first analyses of inorganic constituents of the pulp were made with mature fruits of the Fuerte variety collected at Vista, Calif. The fruits were allowed to soften until edible and were cut into halves along the long axis from stem to tip. There were no consistent differences in the halves, as cut (table 6). The data permit the calculation

of the absolute amounts (grams) of the various ash constituents in fruits of various sizes. A single complete calculation for a Fuerte avocado fruit of a given weight is also presented in table 6.

TABLE 6.—*Mineral content, on a dry-matter basis, of pulp (without skin) of Fuerte avocado fruits, halved lengthwise from stem to tip*

Fresh weight			Dry matter	Ash on dry-matter basis	Ca	Mg	K	Na	Inorganic PO_4	Cu	Fe	Mn
Skin (all)	Seed	Pulp halves										
Grams	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Parts per million*	*Parts per million*	*Parts per million*
31	59	168.5	31.47	3.89	0.025	0.095	1.80	0.43	.....	.....	.....	.....
		155.0	31.18	4.22	.023	.097	2.01	.44	.....	.....	.....	.....
26	59	149.0	34.33	4.18	.018	.104	2.01	.47	.....	.....	.....	.....
		135.8	34.43	4.25	.020	.100	2.02	.50	.....	.....	.....	.....
28	34	145.0	29.07	4.75	.023	.116	2.21	.53	.....	.....	.....	.....
		172.0	29.20	4.48	.025	.104	2.11	.47	.....	.....	.....	.....
15	71	146.0	34.82	3.68	.....	.....	.....	.....	.....	17.1	.....	.....
		144.0	35.47	3.40	.....	.....	.....	.....	.....	13.4	.....	.....
14.5	60	143.5	34.98	3.47	.....	.....	.....	.....	.....	14.7	.....	.....
		145.0	34.25	3.74	.....	.....	.....	.....	.....	14.7	.....	.....
14.25	59	141.3	34.10	3.87	.023	.100	1.63	.23	0.34	.....	44	4.9
		154.0	34.11	3.97	.022	.097	1.63	.23	.29	.....	32	5.2
15	74	186.5	31.06	4.52	.025	.110	2.04	.08	.26	.....	28	5.0
		181.5	32.33	3.95	.038	.099	1.83	.16	.54	13.0	46	4.7
13.5	52.5	143.5	31.23	3.91	.064	.218	1.77	.12	.32	.....	29	4.9
		141.0	31.75	3.96	.032	.103	1.81	.15	.33	.....	36	5.4
13.5	81.5	188.0	31.28	3.99	.033	.094	1.83	.16	.54	14.8	46	4.7
		193.5	31.40	3.93	.044	.091	1.77	.13	.52	15.6	42	3.9
										Milligrams	*Milligrams*	*Milligrams*
			Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*			
[1] 13.5	[1] 81.5	188.0	58.8029	2.3446	0.0195	0.0555	1.0748	0.0915	0.3111	0.873	2.68	0.275
		193.5	60.7500	2.3879	.0270	.0555	1.0748	.0785	.3140	.947	2.56	.235

[1] Absolute amounts of ash constituents given for this sample.

Fruits of the Fuerte, Puebla, and Benik varieties collected at various stages of development were cut transversely into stem and tip halves immediately after being picked. The results of analyses of the pulp of these fruits are presented in a series of graphs.

The rate at which the dry matter of the fresh pulp changes with increasing maturity is shown in figure 1, *A*. As the fruits approach maturity (Fuerte and Benik varieties) there is a more or less abrupt change in the slope of the curves. The maximum dry matter in the pulp of Fuerte avocado fruits was approximately 37 percent. In most of the samples the tip halves of the pulp contain a somewhat larger percentage of dry matter than the stem halves. These results not only confirm the data given in table 1 but indicate also that this same condition in the halves exists more or less generally throughout the various stages in the development of the fruit. The percentages of dry matter show large differences in the inner and outer portions of the tip halves of the pulp of Fuerte avocado fruits.

The ash as a percentage of the dry matter of the tip halves of the pulp of Fuerte and Benik avocado fruits is greater than that of the stem halves (fig. 1, *B*). The graphs for the pulp of fruit of these and other varieties not presented confirm and greatly extend the results of Church and Chace (*1*).

Potassium is by far the most abundant constituent of the ash. Potassium occurs in greater concentration in the dry matter of the tip than in that of the stem halves throughout the various stages of development of the fruit (fig. 2, *A*). In figure 2, *A*, some of the curves

also show the relative uniformity of the percentages of potassium in the pulp of fruits of different ages; in other curves a decreasing trend is shown.

The percentages of sodium in the dry matter are small. The curves shown in figure 2, *B*, indicate higher percentages of sodium as the fruits develop.

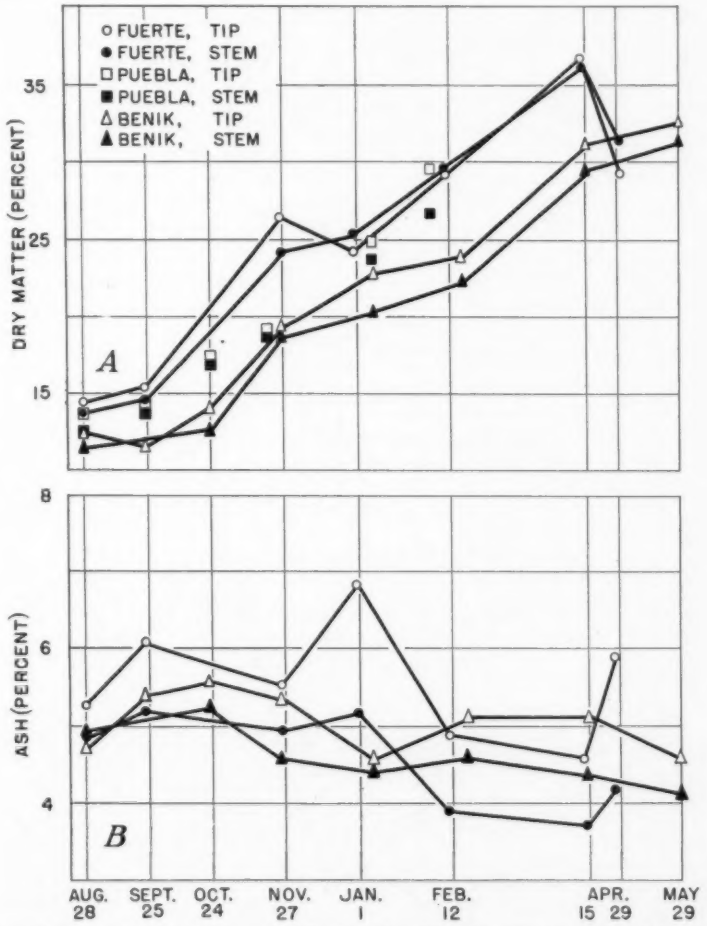

FIGURE 1.—Dry matter (*A*) and ash content (*B*) of the pulp, without skin, from the stem and tip halves of avocados at various stages of development.

The percentage of calcium in the dry matter of the pulp of fruits of the Fuerte, Benik, and Puebla varieties decreases as their season of growth advances (fig. 3, *A*). The stem halves of the pulp contain greater percentages of calcium in the dry matter than the tip halves (fig. 3, *A*), whereas the converse is true in the case of potassium (fig. 2, *A*).

The percentages of magnesium in the stem halves of the pulp are generally greater than those in the tip halves (fig. 3, *B*). The data for magnesium resemble those for calcium, the percentages of magnesium decreasing as the fruits increase in age.

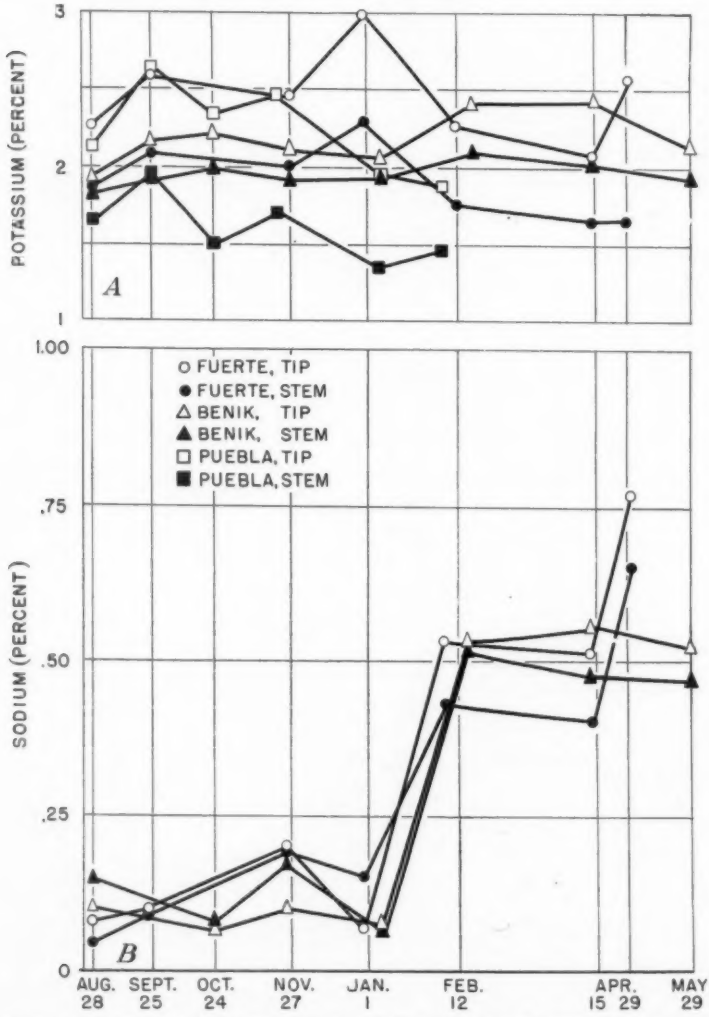

FIGURE 2.—Potassium (*A*) and sodium (*B*) content, on a dry-matter basis, of the pulp, without skin, from the stem and tip halves of avocados, at various stages of development.

The inorganic phosphate as a percentage of the dry matter of the pulp decreases with increasing age of the fruits. The percentages of inorganic phosphate were not uniformly greater in the tip than in the stem halves of pulp (fig. 4). Inorganic phosphate as a percentage of

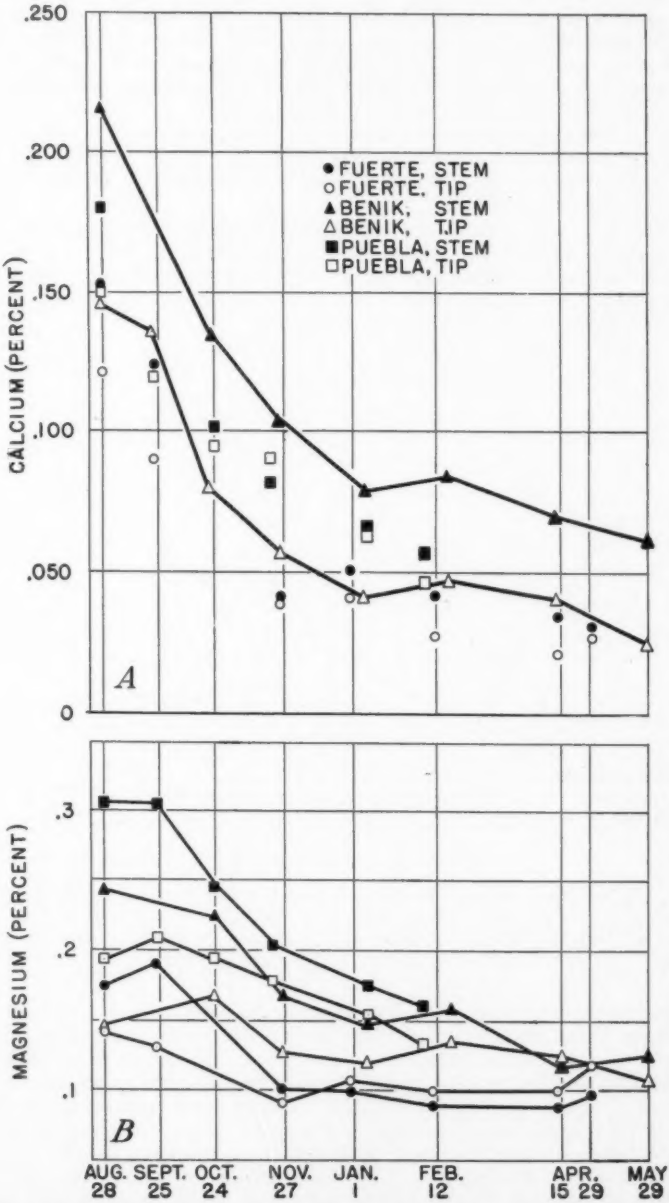

FIGURE 3.—Calcium (*A*) and magnesium (*B*) content, on a dry-matter basis, of the pulp, without skin, from the stem and tip halves of avocados, at various stages of development.

the dry matter of the pulp was somewhat greater in the tip than in the stem halves of Blake avocado fruits. The values for the stem halves were 0.76, 0.82, and 0.66, while those for the tip halves were 0.80, 0.84, and 0.74 for the respective samples (table 7) collected on August 28, September 15, and October 10, 1930. There are no known available data regarding the iron and manganese content of avocado fruits. Data regarding the iron and manganese concentrations in the pulp are presented in table 7. The variations in the values obtained for iron are large and the differences may be merely fortuitous and statistically not significant.

Except in the small fruits, which were of the Blake variety, the manganese content (p. p. m.) in the tip halves of the pulp of the fruits examined exceeds that in the stem halves (table 7). Figure 5 shows that the concentration of manganese decreases as the fruits become older.

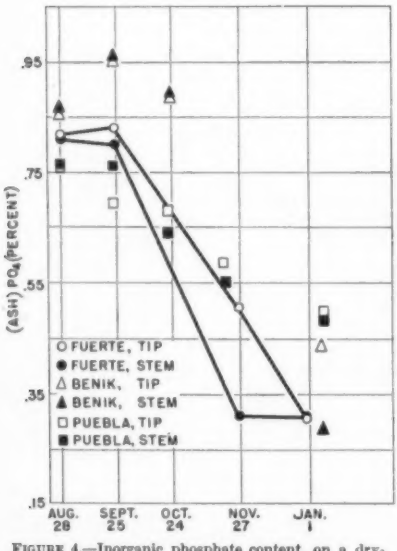

FIGURE 4.—Inorganic phosphate content, on a dry-matter basis, of the pulp, without skin, from the stem and tip halves of avocados, at various stages of development.

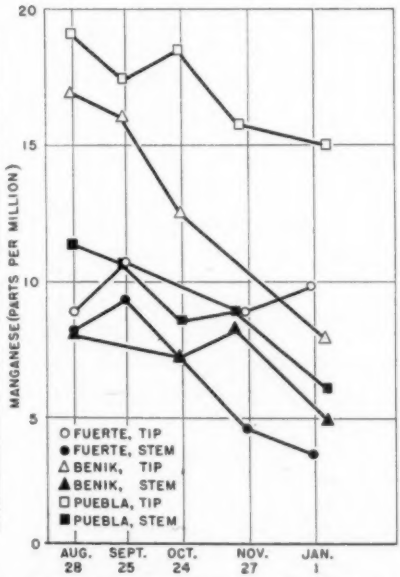

FIGURE 5.—Manganese content, on a dry-matter basis, of the pulp, without skin, from the stem and tip halves of avocados, at various stages of development.

Because of the extremely large number of commercial varieties of avocado fruits, some of the ash constituents of additional varieties were also determined (table 8). Except in the first two samples of Blake avocado fruits, the percentages of calcium are again larger in the stem than in the tip halves. No definite trend was shown by the values for magnesium or sodium, whereas in every case the percentage of potassium in the tip halves exceeds that in the stem halves.

No data are available in the literature in regard to the copper content of avocado fruits. Determinations were made of this constituent in the pulp and skin of mature fruits of the Anaheim variety

collected at Fallbrook in June 1933. The limited data (table 9) indicate that the greatest copper content in the pulp was found in the outer portion of the tip half. Large amounts of copper also appear to be present in the skin of the fruit. The variation in the results for the inorganic phosphate content of the two lots of fruit was extremely large.

TABLE 7.—*Iron and manganese content, on a dry-matter basis, of the pulp (without skin) from the stem and tip halves of avocado fruits of several varieties*

Variety and date of collection	Fruits	Iron		Manganese	
		Stem half	Tip half	Stem half	Tip half
	Number	*Parts per million*	*Parts per million*	*Parts per million*	*Parts per million*
Fuerte:					
Aug. 28, 1930	4	67	26	8.3	8.9
Sept. 23, 1930	7	41	10	9.4	10.7
Nov. 26, 1930	8	41	50	4.6	8.8
Dec. 29, 1930	10	32	28	3.7	9.8
Puebla:					
Aug. 28, 1930	5	23	119	11.4	19.1
Sept. 23, 1930	8	15	28	10.7	17.5
Oct. 23, 1930	10	65	27	8.5	18.5
Nov. 21, 1930	10	19	79	6.8	15.8
Jan. 6, 1931	12	20	51	6.0	15.0
Puebla seedling:					
Sept. 3, 1930	16	32	60	5.8	7.8
Oct. 10, 1930	10	16	18	4.2	6.1
Nov. 21, 1930	4	19	51	4.1	5.5
Benik:					
Aug. 28, 1930	4	30	54	8.0	17.0
Sept. 23, 1930	7	----------	34	----------	16.1
Oct. 24, 1930	8	16	22	7.3	12.5
Nov. 26, 1930	8	55	----------	8.1	----------
Jan. 6, 1931	9	36	48	4.9	7.9
Blake:					
Aug. 28, 1930	5	64	40	10.8	10.2
Sept. 15, 1930	6	73	23	10.0	9.6
Oct. 10, 1930	10	59	54	8.5	8.8

TABLE 8.—*Inorganic base content, on a dry-matter basis, of the pulp (without skin) from the stem and tip halves of avocado fruits of several varieties*

Variety [1]	Fruits	Ash		Calcium		Magnesium		Potassium		Sodium	
		Stem half	Tip half	Stem half	Tip half	Stem half	Tip half	Stem half	Tip half	Stem half	Tip half
	Number	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Premier	3	5.93	6.49	0.084	0.059	0.166	0.186	2.60	2.96	0.10	0.13
Lyon seedling	5	5.08	5.55	.064	.044	.148	.131	2.35	2.56	.06	.09
Thompson	3	4.07	5.27	.082	.031	.160	.158	1.84	2.36	.10	.09
Anaheim	4	6.66	7.92	.076	.046	.169	.211	2.92	3.70	.30	.17
Blake	5	4.77	5.83	.045	.050	.154	.132	2.16	2.48	.09	.08
Do	6	5.16	6.18	.047	.073	.129	.159	2.34	2.87	.13	.15
Do	10	3.99	4.90	.040	.027	.115	.119	1.79	2.27	.06	.06

[1] Samples of the fruits of the Blake variety were collected respectively on Aug. 28, Sept. 15, and Oct. 10, 1930; those of the other varieties were collected on Mar. 12, 1930.

The pulp of mature Fuerte, Blake, Puebla, and Benik avocado fruits was next investigated to find any possible differences in the total sulphur, phosphorus, nitrogen, and chlorine content in the stem and tip halves. The data for mature fruits are given in table 10.

TABLE 9.—*Copper and inorganic phosphate content, on a dry-matter basis, of the pulp and skin from the stem and tip halves of Anaheim avocado fruits, 1933*

Part of fruit and date of collection	Fruits	Copper				Inorganic phosphate			
		Inner stem	Outer stem	Inner tip	Outer tip	Inner stem	Outer stem	Inner tip	Outer tip
Pulp:	*Number*	*Parts per million*	*Parts per million*	*Parts per million*	*Parts per million*	*Percent*	*Percent*	*Percent*	*Percent*
June 14	1	30.3	22.0	26.6	34.1	0.894	0.780	0.760	0.885
June 23	2	28.3	23.4	23.6	36.6	.398	.336	.328	.378
Skin:[1]									
June 14	1	32.1	32.1	44.2	44.2	.396	.396	.638	.638
June 23	2	26.7	26.7	19.4	19.4	.659	.659	.556	.556

[1] The skin was separated into stem and tip but not into inner and outer portions, hence the figures are given as the same for both the latter.

TABLE 10.—*Total sulphur, phosphorus, nitrogen, and chlorine content, on a dry-matter basis, of the pulp (without skin) from the stem and tip halves of avocado fruits of several varieties in relation to time of collection*

Variety and date of collection	Fruits	Sulphur		Phosphorus		Nitrogen		Chlorine	
		Stem half	Tip half	Stem half	Tip half	Stem half	Tip half	Stem half	Tip half
Fuerte:	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Nov. 26, 1930	8	0.13	0.13	0.13	0.12	1.28	1.43	0.02	.....
Dec. 29, 1930	10	.14	.15	.41	.10	1.30	1.51	.03	0.03
Dec. 30, 1930	10	.16	.21	.11	.14	1.28	1.65	.....	.....
Feb. 10, 1931	10	.10	.11	.11	.34	.77	.97	.03	.03
Apr. 13, 1931	10	.10	.11	.14	.15	.71	.89	.02	.02
Apr. 30, 1931	6	.12	.14	.11	.10	1.03	1.32	.02	.02
Blake:									
Oct. 10, 1930	10	.10	.11	.11	.12	.89	1.03	.05	.09
Puebla:									
Oct. 23, 1930	10	.12	.13	.11	.....	.....	.....	.....	.....
Nov. 21, 1930	10	.12	.12	.11	.12	1.51	1.71	.....	.....
Jan. 6, 1931	12	.08	.10	.11	.10	1.08	1.27	.04	.04
Feb. 4, 1931	10	.08	.08	.11	.11	.91	1.03	.03	.03
Benik:									
Jan. 6, 1931	9	.10	.09	.10	.10	1.21	1.29	.01	.01
Feb. 16, 1931	8	.09	.09	.09	.18	.99	1.16	.02	.02
Apr. 14, 1931	5	.08	.08	.11	.12	.88	.95	.01	.01
May 29, 1931	4	.08	.10	.11	.12	1.01	1.13	.01	.01

The results permit of one definite conclusion, namely, that the percentages of total nitrogen (including nitrates) are greater in the dry matter in the tip than in the stem halves of avocado fruits. This was found to be true in each of the individual sample collections that were made. The dry matter therefore contains higher percentages of both total nitrogen and potassium in the tip than in the stem halves.

The chlorine concentrations in the pulp (table 10) are usually very small. Results on fruits from other locations suggest, however, as will be shown later, that in districts where the irrigation water contains large concentrations of chlorine, higher percentages of total chlorine than those given in table 10 are found. Total sulphur and phosphorus concentrations (table 10) are only about one-tenth that of total nitrogen.

In order to study the effect of high sulphate concentrations in the irrigation water on the total sulphur distribution in avocado fruits, mature fruits [4] of the Challenge, Queen, Spinks, and Taft varieties

[4] These fruits were obtained through the kindness of L. T. Sharp of Santa Paula, Calif.

were obtained at Oxnard, Calif., on September 23, 1933, from trees growing in soil that was irrigated with water containing 350 to 450 parts per million of sulphate. The leaves of the trees from which these fruits were picked were affected with tipburn (*5*).

Although it is highly desirable that the control fruits be of the same variety as, and be collected near, the experimental fruits, it was not feasible in this case to secure such control fruits. Mature fruit samples of the Fuerte and Anaheim varieties (to serve as controls) were obtained on June 15, 1933, at the Citrus Experiment Station, where the sulphate content of the irrigation water is relatively low.

The percentages of total sulphur and phosphorus in the dry matter of the pulp of the control fruits (Fuerte and Anaheim varieties in table 11) are less than those for the fruits obtained from areas high in sulphate. Considerable variation occurred in the percentages of sulphur and phosphorus in the various portions of the pulp and no consistent relation was found for all of the varieties. No consistent relation of total phosphorus to the fruit halves was found in the skin of the fruits of the several avocado varieties used (fig. 6). However, the percentages for total sulphur in the skin (fig. 6) are uniformly greater in the stem than in the tip halves.

TABLE 11.—*Total sulphur and phosphorus content, on a dry-matter basis, of the pulp and skin* [1] *from the stem and tip halves of avocado fruits of several varieties from trees grown in soil containing considerable sulphate, 1933*

[Fuerte and Anaheim grown at Riverside are controls; other varieties grown at Oxnard]

Variety, part of fruit, and date of collection	Sulphur				Phosphorus			
	Inner stem	Outer stem	Inner tip	Outer tip	Inner stem	Outer stem	Inner tip	Outer tip
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Fuerte, pulp, June 15	0.080	0.072	0.073	0.096	0.141	0.104	0.111	0.120
Fuerte, skin, June 15	.139	.139	.077	.077	.243	.243	.120	.120
Anaheim, pulp, June 15	.094	.137	.060	.162	.149	.129	.143	.132
Anaheim, skin, June 15	.058	.058	.049	.049	.208	.208	.206	.206
Challenge, pulp, Sept. 23	.188	.199	.175	.197	.280	.203	.230	.263
Challenge, skin, Sept. 23	.078	.078	.052	.052	.204	.204	.157	.157
Queen, pulp, Sept. 23	.196	.186	.219	.218	.302	.209	.246	.266
Queen, skin, Sept. 23	.050	.050	.042	.042	.156	.156	.160	.160
Spinks, pulp, Sept. 23	.190	.200	.230	.216	.189	.266	.193	.310
Spinks, skin, Sept. 23	.054	.054	.048	.048	.165	.165	.191	.191
Taft, pulp, Sept. 23	.147	.150	.144	.139	.220	.192	.180	.191
Taft, skin, Sept. 23	.067	.067	.057	.057	.163	.163	.166	.166

[1] The skin was separated into stem and tip but not into inner and outer portions hence the figures are given as the same for both the latter.

The pulp of mature avocado fruits secured at Riverside, where the irrigation water contained very little chlorine (27 parts per million), showed very small concentrations of this constituent (table 10). In order to compare these data with those of fruits from areas rich in chlorine, mature fruits were secured from groves at Encinitas, Calif., in June 1933, in which the trees showed severe leaf tipburn as a result of excessive concentrations of chlorine (table 12). The chlorine concentration was determined in the inner and outer portions of the stem and tip halves of the pulp and skin. The fruits were mature, except those of the Itzamna variety, and were allowed to soften before being analyzed.

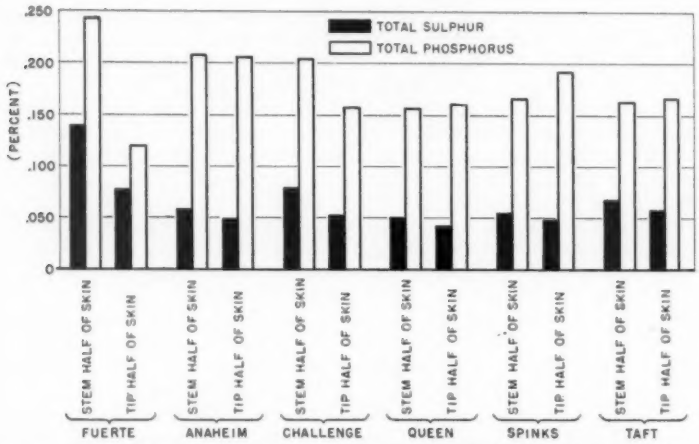

FIGURE 6.—Total sulphur and phosphorus content, on a dry-matter basis, of the skin from the stem and tip halves of six varities of avocado; Fuerte and Anaheim came from trees in control areas and Challenge, Queen, Spinks, and Taft from trees in high-sulphate areas.

The greater amount of chlorine is found in the pulp of fruits from trees bearing leaves which have been injured by chlorides (tables 10 and 12). The pulp and the skin of the tip halves contain smaller percentages of chlorine than the pulp and skin of the stem halves and in each of the varieties studied, the outer portion contains smaller percentages of chlorine than the inner portion of the same halves of pulp (table 12). These results show an inverse relation to potassium.

TABLE 12.—*Total chlorine content, on dry-matter basis, of mature avocado fruits of several varieties obtained from areas containing high chlorine*

Variety	Fruits	Pulp				Skin	
		Stem half		Tip half		Stem half	Tip half
		Inner portion	Outer portion	Inner portion	Outer portion	Entire	Entire
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Anaheim, lot 1	1	0.260	0.200	0.180	0.170	0.380	0.240
Anaheim, lot 2	1	.220	.170	.160	.150	.360	.270
Dickinson	2	.140	.100	.100	.080	.330	.270
Fuerte	3	.084	.060	.049	.037	.780	.770
Itzamna [1]	2	0.073		0.064		.400	.350

[1] Immature fruits.

The results on the chlorine content of the pulp and the skin of mature avocado fruits have been extended to include many varieties growing in soils the solutions of which contain various concentrations of chlorine. The data in table 13 give added support to the view that the skin and the pulp of avocado fruits may accumulate chlorine to a considerable degree when this constituent is present in large concentrations in the soil solution. In general, both the skin and the pulp of the stem halves contain higher percentages of chlorine than the corresponding parts of the tip halves.

TABLE 13.—*Total chlorine content, on a dry-matter basis, of avocado fruits of several varieties from trees grown in soil irrigated with water containing various concentrations of chlorine*

Place and date of collection	Part of fruit and variety	Fruits	Amount of chlorine in irrigation water and extent of tipburn	Cl in—	
				Stem half	Tip half
		Number		*Percent*	*Percent*
Riverside:	Pulp:				
Oct. 23, 1930	Puebla	10	27 parts per million	0.046	0.043
		10		.042	.038
Oct. 24, 1930	Benik	8		.015	.014
Oct. 27, 1930		6		.0122	.0117
Oct. 23, 1930	Fuerte	2		.033	.028
Carlsbad: Dec. 30, 1930		1	Moderate; more than 27 parts per million; no leaf tipburn.	.090	.060
Encinitas: Mar. 26, 1930	do	1	Extreme; bad leaf tipburn; defoliation.	.063	.045
		1		.102	.097
		1		.301	.267
Fallbrook: Mar. 12, 1930	do	5		.148	.127
Riverside:	Skin:				
Mar. 19, 1930	Guatemalan seedling	2	27 parts per million	.031	.024
Feb. 1, 1930	Mayapan	1		.022	.029
Fallbrook: Mar. 27, 1930	Anaheim	6	Moderate; more than 27 parts per million; no leaf tipburn.	.150	.060
	Lyon seedling	7		.130	.090
	Premier	5		.190	.110
	Thompson	6		.135	.096
Near Tustin: Mar. 28, 1930	Challenge	1	High; leaf tipburn	.052	.039
		1		.127	.096
Santa Ana: Feb. 7, 1930	do	1		.178	.144
Near Tustin: Mar. 28, 1930	do	1		.160	.120
Encinitas: June 23, 1933	Fuerte	3		.780	.770
Fallbrook: Mar. 12, 1930		12		.020	.940
Encinitas: Mar. 26, 1930	do	3	Extreme; bad leaf tipburn; defoliation.	1.170	.970
		1		1.280	1.000

When avocado trees are grown under conditions of extremely high chlorine in the soil solution, the percentage of chlorine in the pulp may be increased to about two to seven times that found in fruits grown in districts where the soil solution contains very little chlorine (table 13). The data in table 13 also show the large concentrations of chlorine in the skins of Fuerte fruits from trees grown in soils containing high chlorine. Fuerte skins of this group contained over 1 percent of chlorine in the dry matter, whereas the pulp contained only about 0.1 to 0.3 percent. In the case of Fuerte avocado fruit skins, special care was exercised to remove all traces of pulp from the inside of the thin skin. The results leave no doubt that the high chlorine in the Fuerte skins was actually due to the chlorine in the skins and not to that of adhering pulp, for the pulp alone contained much lower percentages.

These results have not been affected by the adherence of soil, for many of the fruits were high up in the trees and none were allowed to touch the ground. The further precautions of washing the fruits in running, distilled water and drying them with filter paper were used to exclude chlorine contaminations. The high percentages of chlorine in the skins are of interest because as Fuerte fruits become overmature, certain areas of the skin may darken very readily and the tissues die, permitting easy entrance to decay organisms. The writer (*6*) has already described the death of cells surrounding the stomata of mature Fuerte fruits. An excessive accumulation of ions, such as chlorine and potassium, may be responsible in part for skin-tissue break-down,

and hence be related to the problems of storage. The relation of chlorine to break-down of skin tissue in avocado fruits deserves further study.

No analyses of the skin of avocado fruits are at present available other than those just reported. Therefore a study of the concentration of bases in the skin is included in the present investigation.

The percentages of the various bases in the skin of mature avocado fruits of a large number of varieties collected at various locations during 1930–31 are given in table 14. The most striking results are those for calcium, the percentages of which are consistently greater in the dry matter of the skin of the stem half. A second point of interest is that the percentages of ash, calcium, magnesium, sodium, and potassium are greatest in the skin of the fruits of the Fuerte and Benik varieties. The iron content of the dry matter of the tip half of the skin of a fruit (table 9) of the Anaheim variety collected at Fallbrook, on June 23, 1933, was 28 parts per million, and that of whole skins of Fuerte avocado fruits collected at Carlsbad and at Leucadia (table 14) was 248 and 129 parts per million, respectively. The manganese content of the whole skins of Fuerte avocado fruits (table 14) from Carlsbad, Leucadia, and Vista, was 26, 22, and 31 parts per million respectively. The iron content of the skins far exceeds that of manganese. Analyses of the ash of the whole skins of Fuerte avocado fruits from Carlsbad revealed 0.68 percent of inorganic phosphate in the dry matter, while the samples from Leucadia and Vista showed 0.40 and 0.54 per cent, respectively.

TABLE 14.—*Inorganic base content, on a dry-matter basis, of the ash of the skin from the stem and tip halves of avocado fruits of several varieties*

Variety and date of collection	Place of collection	Fruits	Ash		Calcium		Magnesium		Potassium		Sodium	
			Stem half	Tip half	Stem half	Tip half	Stem half	Tip half	Stem half	Tip half	Stem half	Tip half
		No.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Fuerte,[1] Dec. 30, 1930	Carlsbad	2	10.87	10.87	0.200	0.200	0.240	0.240	4.70	4.70	0.52	0.52
Fuerte,[1] Dec. 29, 1930	Leucadia	2	9.36	9.36	.142	.142	.220	.220	4.46	4.46	.45	.45
Fuerte,[1] Jan. 20, 1931	Vista	6	7.67	7.67	.150	.150	.330	.330	3.43	3.43	.34	.34
Do	Puente	6	9.35	9.21	.388	.244	.317	.266	4.26	4.43	2.54	1.09
Fuerte, Mar. 26, 1930	Leucadia	6	8.92	10.03	.238	.117	.291	.293	4.22	4.77	.90	1.27
Benik, May 7, 1930	Riverside	5	9.62	7.52	.704	.196	.239	.248	3.96	3.33	.94	.83
Mayapan, Feb. 1930	do	3	4.30	4.12	.118	.065	.192	.209	1.95	1.88	.48	.45
Mayapan, Mar. 22, 1930	do	5	3.97	3.74	.104	.064	.127	.126	1.85	1.83	.45	.44
Challenge, Mar. 28, 1930	Tustin	6	3.75	3.72	.233	.115	.223	.204	1.07	1.18	.63	.55
Anaheim, Mar. 12, 1930	Fallbrook	6	3.34	2.97	.098	.051	.150	.148	1.40	1.34	.46	.35
Thompson, Mar. 12, 1930	do	6	3.05	3.24	.114	.039	.132	.111	1.38	1.59	.34	.38
Premier, Mar. 27, 1930	do	5	2.82	2.33	.106	.044	.101	.084	1.26	1.10	.29	.25
Premier, Mar. 12, 1930	do	5	2.55	2.19	.220	.062	.142	.085	1.04	.97	.20	.23
Lyon seedling, Mar. 27, 1930	do	7	3.01	2.66	.122	.053	.146	.125	1.29	1.18	.32	.29
Guatemalan seedling R1, T5, Mar. 30, 1930	Riverside	2	3.49	3.06	.118	.063	.114	.114	1.57	1.41	.36	.34
Guatemalan seedling R1, T9, Mar 21, 1930	do	3	2.96	3.43	.127	.056	.121	.151	1.23	1.51	.30	.35
Guatemalan seedling R1, T9, Mar. 18, 1930	do	3	3.09	3.25	.140	.061	.120	.131	1.36	1.52	.33	.37

[1] Whole fruits, unseparated into stem and tip halves, used.

The striking differences in the chemical composition of the skins of avocado fruits are shown in table 14. Certain of the varieties, for example, the Guatemalan seedling, have thick, leathery skins, and a relatively low ash. The Fuerte fruit skin is unique in its high per-

centage of potassium, which frequently is more than twice that found in the pulp. The high percentages of potassium, together with the high chlorine in the skins of fruits from districts that have high chlorine in the soil, suggest possible reasons for the difficulties in handling mature Fuerte fruits.

SUMMARY

The actual acidity in the pulp (without skin) in avocado fruits increases outwardly toward the skin and also increases from the stem toward the tip end, in the inner as well as in the outer portions. This indicates that the outer better aerated tissues are more acid because carbon dioxide, which tends to make the tissues more alkaline, is more quickly removed. The gradients in the acidity may be related to the distribution of stomata in the skin and their distance from the portion of pulp tested.

No consistent differences were found between the fat content of the stem and tip halves of the pulp of avocado fruits.

The pulp (without skin) of the stem halves of avocado fruits contains slightly more reducing and total (as reducing) sugars than that of the tip halves, and in many cases the nonreducing sugar is higher in the tip than in the stem halves. The data indicate a reduction in sugar content with increasing maturity of the fruits. Differences in the content of sugars in the two halves of pulp nearly disappear as maturity is approached.

The halves of pulp of Fuerte avocado fruits cut lengthwise from the stem to the tip end contain approximately equivalent amounts of chemical constituents. Data are given regarding the grams of the various inorganic constituents in a fruit of a given weight.

The tip halves of the pulp (without skin) usually contain in the fresh weight slightly higher percentages of dry matter than the stem halves. As the Fuerte and Benik avocado fruit samples approach maturity, a somewhat marked change occurs in the slope of the curves for the dry-matter percentages.

The ash as a percentage of the dry matter of the tip halves of the pulp of Fuerte avocado fruits is greater than that of the stem halves.

Potassium is the most abundant constituent of the ash and occurs in greater concentration in the tip halves of the pulp of Fuerte avocado fruits than in the stem halves.

The sodium content of the pulp is small but the percentages in the dry matter increase as the fruits develop.

The percentages of calcium in the pulp of Fuerte avocado fruits are small. The stem halves of the pulp contain greater percentages of calcium in the dry matter than the tip halves. The direction of the calcium gradient in the pulp halves is opposite to that of potassium.

The inorganic phosphate, as a percentage of the dry matter, decreases with increasing age of the fruits.

Data are given regarding the iron and manganese content of the various portions of the pulp. The differences in the iron content of fruit halves do not appear to be statistically significant. The concentrations of manganese in the tip halves of the pulp of fruits of the Fuerte, Puebla, and Benik varieties exceed those in the stem halves. The manganese content in the fruit halves decreases as the fruits become older.

Both the skin and pulp of Anaheim avocado fruits contain relatively large concentrations of copper in the pulp and skin. The largest copper content in the pulp occurs in the outer portion of the tip half.

The percentages of total nitrogen (including nitrates) are greater in the dry matter in the tip than in the stem halves of avocado fruits.

The percentages of total sulphur and total chlorine are greater in the stem than in the tip halves of the dry matter of the skin of avocado fruits.

The pulp and the skin of the tip halves of avocado fruits, grown under conditions of high chlorine, contain smaller percentages of chlorine than the pulp and the skin of the stem halves. In each of the varieties studied, the outer portion contains smaller percentages of chlorine than the inner portion of the same halves of pulp. The skin and pulp of avocado fruits may accumulate chlorine to a considerable degree when chlorine is present in large concentrations in the soil solution. It is possible that chlorine may be involved in the break-down of tissue in the skin of overmature avocado fruits.

The percentages of calcium in the dry matter of the skin of avocado fruits are greater in the stem than in the tip halves. Higher percentages of ash, calcium, magnesium, sodium, and potassium are found in the skin of fruits of the Fuerte and Benik varieties than in that of fruits of several other varieties studied.

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ASCORBIC ACID CONTENT OF A NUMBER OF CITRUS FRUITS[1]

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INTRODUCTION

Among a number of citrus hybrids that have been developed by the Bureau of Plant Industry, United States Department of Agriculture, are the tangelo, a grapefruit and tangerine cross, the tangor having an orange and tangerine parentage, the limequat and orangequat, crosses of the lime and tangerine, respectively, with the kumquat, and the Perrine lemon, a lemon-lime hybrid.

Because of the prominence many citrus fruits have gained by reason of their high ascorbic acid content, it was considered important to determine the ascorbic acid values of some of these new citrus hybrids for comparison with the values for the more common citrus fruits.

A description of most of the hybrid fruits that were tested has been given elsewhere by Swingle, Robinson, and Savage.[3] In addition to the information about the vitamin C content of commercial types, this work will indicate the capacity for inheritance of this vitamin. The work is being continued on a cooperative basis between the two Bureaus concerned.

References to ascorbic acid values for a limited number of varieties of oranges, grapefruit, tangerines, lemons, and limes, may be found among the vitamin data reviewed by Daniel and Munsell.[4]

MATERIALS AND METHODS

The fruits were selected and shipped to Washington from the United States Horticultural Field Laboratory, Orlando, Fla., by members of the Bureau of Plant Industry. Except for two samples of Perrine lemons, which were picked in different stages of ripeness (ripe yellow and light green) and kept in cold storage 2 months before shipping, all of the samples represent freshly picked fruit. Analyses for ascorbic acid were made as soon as possible after the arrival of the fruit in Washington.

The first shipments contained a number of different fruits, among which were oranges, grapefruit, tangerines, tangelos, lemons, limes, limequats, and orangequats. These were received between December 20 and 27, 1935, and the analyses were made within the following 2 weeks. The ascorbic acid content of one sample of each of these fruits

[1] Received for publication Jan. 7, 1937; issued June 1937.
[2] Appreciation is expressed to H. P. Traub, T. R. Robinson, and E. M. Savage, Division of Fruit and Vegetable Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, for their cooperation in selecting and sending representative samples of fruit.
[3] SWINGLE, W. T., ROBINSON, T. R., and SAVAGE, E. M. NEW CITRUS HYBRIDS. U. S. Dept. Agr. Circ. 181, 20 pp., illus. 1931.
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was determined. Some of the most promising varieties, selected by members of the Bureau of Plant Industry on the basis of fruits showing the greatest commercial possibilities, were tested at weekly intervals over a period of 6 weeks. A group of five varieties of these selected fruits, including Bowen grapefruit, Dancy tangerine, Minneola and Thornton tangelos, and Pineapple orange, were tested between February 2 and March 13, 1936. Five other varieties, including Davis and Foster (pink) grapefruit, Seminole tangelo, Umatilla tangor, and Valencia oranges were analyzed between March 20 and April 24, 1936.

The ascorbic acid measurements were carried out essentially according to the titration method described by Bessey and King.[5] The sample was prepared by hand-reaming several fruits and lightly shaking the resultant juice and pulp through an 8-mesh wire sieve.[6] After thorough mixing, a 10-cc aliquot of this juice was pipetted into a centrifuge tube, 25 cc of hot 8-percent acetic acid added, and the whole centrifuged for 5 minutes. After decanting into a 100-cc volumetric flask, 10 cc more of the hot 8-percent acetic acid was added to the residue, the sides of the tube were washed down with approximately 25 cc of water distilled from glass, and the mixture centrifuged for 3 minutes. After decanting, 10 cc more of the hot acid was added and the above procedure again repeated, this time centrifuging for 2 minutes. The combined supernatant liquids were made up to 100 cc, thoroughly mixed, and three 10-cc aliquots, each equivalent to 1 cc of the original juice, were titrated with a standardized solution of 2, 6 dichlorophenolindophenol. Two separate determinations using threefold checks on the titration procedure were made on each sample of fruit. Blanks were carried through with the earlier experiments until it was found that they consistently showed no titration value, after which they were discontinued.

The 2, 6 dichlorophenolindophenol was made up in 0.02-percent solution, filtered and titrated against a commercial ascorbic acid, also made up in 0.02-percent solution. This commercial preparation of ascorbic acid was found to have the same titration value as the International Standard of ascorbic acid. The ascorbic acid solutions were titrated immediately after preparation. The 2, 6 dichlorophenolindophenol was prepared in this laboratory according to the directions given by Bessey and King.[7] Solutions of this dye were made up each day and standardized against freshly prepared ascorbic acid solutions containing a known weight of the acid. Freshly boiled water previously distilled from glass was used for all solutions. The titrations were made as rapidly as possible and the first faint pink color was taken as indication that the end point had been reached. All of the citrus fruits tested gave a sharp end point which was readily determined. The ascorbic acid content of the samples was calculated in terms of milligrams of ascorbic acid per cubic centimeter of expressed fruit juice.

RESULTS

The average ascorbic acid values for a large number of citrus fruits are brought together in table 1. Included in this list are: The isolated

[5] BESSEY, O. A., and KING, C. G. THE DISTRIBUTION OF VITAMIN C IN PLANT AND ANIMAL TISSUES, AND ITS DETERMINATION. Jour. Biol. Chem. 103: 687-698. 1933.

[6] Olive McElroy, of this laboratory, has shown that juice strained in this manner gives the same ascorbic acid titration as juice squeezed through muslin.

[7] BESSEY, O. A., and KING, C. G. See footnote 5.

single samples of several hybrids; a number of the more common citrus fruits, including hybrid-parent fruits; other fruits which were analyzed at weekly intervals, and are reported in detail in table 2; and also several varieties of oranges tested in the course of a previous experiment [8], the results being recorded here for purposes of comparison. These last are all designated in the table as "commercial shipments." All other samples were obtained through the United States Horticultural Field Laboratory, Orlando, Fla. The soil conditions in which the different fruits were grown have been indicated in all cases in which this was known.

The individual and the average ascorbic acid values for six samples each of several varieties of citrus fruits tested at weekly intervals are given in table 2.

TABLE 1.—*Ascorbic acid content of a number of miscellaneous samples of citrus fruits*

Fruit tested	Variety	Origin of fruit and description of soil or fruit	Samples tested (2 determinations per sample)	Fruits per sample	Average ascorbic acid per cubic centimeter of juice
			Number	*Number*	*Milligram*
		Merritt's Island, Indian River section, Fla., hammock soil (sandy loam) underlaid with marl subsoil.	1	5	0.62
	Pineapple	Florida, commercial shipments from Orlando locality during 2½ month period, Jan. 9, 1936, to Mar. 20, 1936.	9	6	.51
		Eustis, Fla., high pine land, Norfolk sand, clay subsoil, typical of Lake County citrus district.	1	6	.51
			6	5	.49
	Valencia	Florida, commercial shipments from Orlando locality during 1½-month period, Mar. 13, 1936, to Apr. 24, 1936.	7	6	.45
Orange (*Citrus sinensis*).		California, commerical shipment from various parts of State during 3½-month period, Aug. 29, 1935, to Dec. 6, 1935.	16	6	.40
		Eustis, Fla., high pine land, Norfolk sand, clay subsoil, typical of Lake County citrus district.	6	5	.32
	Washington Navel.	California, commercial shipments from various parts of State during 3½-month period, Dec. 27, 1935, to Apr. 10, 1936.	15	6	.58
	Sanford Mediterranean.	Eustis, Fla., high pine land, Norfolk sand, clay subsoil, typical of Lake County citrus district.	1	6	.51
	Hamlin		1	3	.48
	Majorca		1	6	.43
	Homosassa		1	6	.43
	Ruby (Blood)		1	6	.37
	Tresca [1]		1	2	.64
Grapefruit (*Citrus grandis*).	Davis		1	3	.38
	do	do	6	2	.34
	Bowen		6	2	.35
	Foster (pink)		6	2	.33
	Clementine [2]		1	6	.37
Tangerine (*Citrus nobilis deliciosa*).	Dancy	do	6	5–6	.24
	do	Orlando, Fla., sandy loam soil.	1	6	.18

[1] Belongs to shaddock group.
[2] Believed to be a natural hybrid of tangerine and sour orange.

[8] DANIEL, E. P., KENNEDY, M. H., and MUNSELL, H. E. RELATIVE VITAMIN C CONTENT OF ORANGE AND TOMATO JUICES DETERMINED CHEMICALLY AND BIOLOGICALLY. Jour. Home Econ. 28: 470–474. 1936.

TABLE 1.— *Ascorbic acid content of a number of miscellaneous samples of citrus fruits*—Continued

Fruit tested	Variety	Origin of fruit and description of soil or fruit	Samples tested (2 determinations per sample)	Fruits per sample	Average ascorbic acid per cubic centimeter of juice
			Number	*Number*	*Milligram*
	Clement [2]	Eustis, Fla., high pine land, Norfolk sand, clay subsoil, typical of Lake County citrus district.	1	[4] 3	0.64
	Thornton	Merritt's Island, Indian River section, Fla., hammock soil (sandy loam) underlaid with marl subsoil.	1	2	.43
	Wekiwa	Eustis, Fla., high pine land, Norfolk sand, clay subsoil, typical of Lake County citrus district.	1	[5] 6	.43
	Orlando		1	5	.35
Tangelo (*Citrus nobilis deliciosa* × *C. grandis*).	Thornton		6	5–6	.33
	Sunshine	Vicinity of Vero Beach, Indian River section, Fla., New Hybrid No. 52018–K–12 (quite soft when tested).	1	1	.29
	Sampson	Merritt's Island, Indian River section, Fla., hammock soil (sandy loam) underlaid with marl subsoil.	1	2	.28
	Minneola		1	[4] 2	.27
	....do	Eustis, Fla., high pine land, Norfolk sand, clay subsoil, typical of Lake County citrus district.	6	4–5	.22
	....do		1	[6] 4	.22
	Seminole		6	4	.18
Tangor (*Citrus nobilis* × *C. sinensis*).	Umatilla	....do	6	3	.40
Lemon (*Citrus limonia*).	Sweet		1	7	.33
Lemon-lime hybrid (*Citrus aurantifolia* × *C. limonia*).	Perrine	Eustis, Fla., high pine land, Norfolk sand soil, clay subsoil, typical of Lake County citrus district: Fresh picked fruit	1	6	.40
	....do	Picked in ripe-yellow stage and stored (cold storage) Oct. 23 to Dec. 19, 1935.	1	10	.24
	....do	Picked in light-green stage and stored (cold storage) Oct. 23 to Dec. 19, 1935.	1	8	.26
Lime (*Citrus aurantifolia*).	West Indian or Key.	Orlando, Fla., Orlando sandy loam soil.	1	14	.22
Limequat (*Citrus aurantifolia* × *Fortunella japonica*).	Lakeland	Lake Alfred, Fla., substation, Norfolk sand, orange-colored clay subsoil typical of main Ridge citrus district.	1	12	.17
Orangequat (*Citrus nobilis* × *Fortunella* spp.).	Nippon	Eustis, Fla., high pine land, Norfolk sand, clay subsoil, typical of Lake County citrus district.	1	8	.23

[2] Hybrid of clementine tangerine with grapefruit; other tangelos had Dancy tangerine as pollen parent.
[4] Large. [5] Small. [6] Medium-sized.

TABLE 2.—*Ascorbic acid content of certain varieties of grapefruit, tangerines, tangelos, and oranges*

[Milligrams per cubic centimeter of juice]

Sample no.[1]	Grapefruit			Tangerine	Tangelo			Tangor	Orange	
	Bowen, 2 fruits per sample	Davis, 2 fruits per sample	Foster (pink), 2 fruits per sample	Dancy, 5–6 fruits per sample	Minneola, 4–5 fruits per sample	Thornton, 5–6 fruits per sample	Seminole, 4 fruits per sample	Umatilla, 3 fruits per sample	Pineapple, 5 fruits per sample	Valencia, 5 fruits per sample
	Milligram	*Milligram*	*Milligram*	*Milligram*	*Milligram*	*Milligram*	*Milligram*	*Milligram*	*Milligram*	*Milligram*
1	0.38	0.33	0.31	0.20	0.19	0.31	0.20	0.37	0.42	0.33
2	.33	.34	.32	.27	.23	.29	.20	.37	.48	.33
3	.34	.34	.35	.24	.22	.35	.20	.43	.50	.33
4	.35	.34	.34	.24	.19	.30	.15	.36	.50	.32
5	.33	.34	.32	.25	.23	.34	.17	.35	.54	.30
6	.34	.33	.34	.22	.27	.37	.16	.53	.53	.32
Average	.35	.34	.33	.24	.22	.33	.18	.40	.49	.32

[1] Each sample represents a weekly shipment of the different varieties of fruits which were tested.

SUMMARY AND CONCLUSIONS

Ascorbic acid values were determined for the following freshly picked citrus fruits: Eight varieties of oranges, three varieties of grapefruit, two varieties of tangerines, eight varieties of tangelos (a grapefruit-tangerine hybrid), one variety each of tangor (a tangerine-orange hybrid), lemons, limes, limequats (a lime-kumquat hybrid), orangequats (a tangerine-kumquat hybrid), and the Perrine lemon (a lemon-lime hybrid).

It was found that the ascorbic acid values of the juices of the eight varieties of oranges varied within the limits of 0.32 and 0.62 mg per cubic centimeter of juice. All the varieties of grapefruit tested except for the one sample of Tresca variety had ascorbic acid values in the region of the lower values found for the oranges. The average ascorbic acid values of six samples of Umatilla tangor was 0.40 mg per cubic centimeter of juice, a value slightly higher than the average value for all varieties of grapefruit and somewhat lower than that for most of the varieties of oranges tested.

Only a few samples of tangerines, limes, limequats, and orangequats were tested, but all of these carried relatively small concentrations of ascorbic acid, on the order of half the concentration of that found in most of the varieties of oranges that were analyzed.

The ascorbic acid content of the juices of eight varieties of tangelos (a grapefruit-tangerine hybrid) ranged all the way from the highest value found for grapefruit to the lowest value for tangerines.

Preliminary tests on Perrine lemons picked both in the ripe-yellow and light-green stages, and kept for 2 months in cold storage, indicated that the ascorbic acid was approximately the same for both. This was about 60 percent of the amount originally found to be present in the ripe, freshly picked fruit.

ANATOMY AND HISTOLOGY OF THE TRANSITION REGION IN CAPSICUM FRUTESCENS[1]

By H. L. COCHRAN, *associate horticulturist* and F. F. COWART, *assistant horticulturist, Georgia Experiment Station*

INTRODUCTION

The Solanaceae family has received special attention in the past from the standpoints of both physiology and anatomy. The anatomical variations of the plants in this family are fairly well known, nevertheless numerous details are yet to be determined for the plants within the genera and species. It was the purpose of this investigation to depermine the method of root-stem transition in *Capsicum frutescens* Linn., and to note the similarity, if any, to that of some other closely related species of solanaceous plants, several of which have been reported.

REVIEW OF LITERATURE

As far as the authors were able to determine the botanical literature reveals no reports dealing with the anatomy of the transition region in *Capsicum*.

Von Hanstein (*4*)[2] in 1864 first reported the occurrence of internal phloem in the Solanaceae, but it was not until 1875 that Vesque (*8*) described its distribution. Petersen (*5*) in 1882 not only confirmed the findings of Vesque but extended them to several different genera of the family as well. Weiss (*9*), however, in 1883 published what appears to be the first detailed histological discussion of the Solonaceae.

Artschwager (*1*) in studying the method of root-stem transition in the potato plant reported that, in the change from the exarch condition of the root to the endarch condition of the stem, the two protoxlyem groups of the root swing outward, one group following a left the other a right curve. He reported also that the primary xylem groups assume a position in a tangential plane instead of forming a radial row in the portion of the hypocotyl just beneath the cotyledons. The above-mentioned change from the exarch to the endarch condition was found to be completed above the cotyledons.

King, as reported by Thiel (*6*), studied the root-stem transition in *Lycopersicum esculentum* Mill, and found that the metaxylem was differentiated tangentially toward the periphery of the stele at successively higher levels. It was also found that the protoxylem maintained its original position until a point was reached just below the cotyledonary plate. Centripetal differentiation of the protoxylem started at this point and continued until the primary xylem groups were almost endarch at the cotyledonary plate. It was also reported that the primary phloem divided into smaller groups, and that strands which were differentiated toward the center constituted the internal phloem. The external phloem formed four equally distributed groups, which were oriented collaterally at the outer face of the

[1] Received for publication Dec. 12, 1936; issued June, 1937. Paper No. 48, Journal series of the Georgia Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 700.

xylem groups. Simultaneously the internal phloem was found to form two groups located at the inner limits of the primary xylem, thus establishing the bicollateral condition.

Thiel (*6*) investigated the root-stem transition of *Solanum melongena* Linn., and found it to begin very low in the hypocotyl, occurring approximately 2.25 cm below the cotyledons. The first change noted was a breaking up of the diarch xylem plate and the two primary phloem groups forming two units of primary xylem and phloem. Subsequently a bifurcation of the two phloem groups was found to occur at a level slightly higher than where the root-stem transition was first noted. The bicollateral condition was found to be established by an inward differentiation and division of several small primary phloem groups that come to lie opposite the inner faces of the primary xylem elements. The four remaining groups lying nearest the metaxylem were found gradually to become inclined in a tangential direction, eventually lying on the outside of the original position of the protoxylem points. This complete change from the exarch condition of the root to the endarch condition of the stem was found to take place in the midrib of the cotyledon.

Clark (*2*) in studying the stem anatomy of tomato, *Lycopersicum esculentum*, devoted special attention to the internal phloem. The conclusion was reached that strands of the external phloem move inward during the transition period and become oriented at the center or base of the xylem elements. A division of the phloem cells themselves was thought to be responsible for the additional internal phloem.

Thiel (*7*) in his study of the anatomy of the primary axis of *Solanum melongena* found that the method of root-stem transition was that reported by King for the tomato, but his results were not in agreement with those of Artschwager for the potato plant. For this reason the vascular anatomy of the transition region of certain other solanaceous plants was investigated among which were included *Solanum tuberosum* Linn, *Lycopersicum esculentum*, *Datura tatula* Linn., *Physalis virginiana* Mill, and *S. pseudo-capsicum*, Linn. The method of transition in each of these plants was found to be the same, and was in all cases identical with that of *S. melongena*.

Woodcock (10) investigated the vegetative anatomy of the tomato and found that the structure of the stem was quite similar to that of the potato stem as described in detail by Artschwager, but nothing was reported in this work relative to the method of root-stem transition.

MATERIALS AND METHODS

The Perfection variety of pimiento alone was used in this study. All plants were grown in pots in the horticultural greenhouse of the Georgia Experiment Station during the spring and summer of 1936. Seedlings ranging from 1 to 27 days old were used, but those ranging in age from 5 to 7 days furnished the best material for a detailed analysis of the root-stem transition. At this age the primary tissues were practically completely differentiated but little or no secondary growth had taken place.

The material was killed and fixed in Karpechenko's chromacetic solution (solutions A and B being made up separately and then mixed in equal parts at the time of use), dehydrated in alcohol and embedded in paraffin using xylol. Transverse and longitudinal sections were

cut 10μ thick, mounted serially on slides, and stained with Heidenhain's iron-alum haematoxylin and safranin.

RESULTS

GROSS MORPHOLOGY OF THE YOUNG SEEDLING

The first external indication of germination is a slight swelling of the seed coat and soon thereafter an elongation of the radicle from the micropyle (fig. 1, *a*, *b*). When the radicle is 8 to 10 mm in length the small growing hypocotyl assumes a curved position and within 4 or 5 days, depending upon the temperature, appears above the soil in the form of a closed loop (fig. 1, *e*). The elongation of the hypocotyl brings the cotyledons, which are normally still enclosed by the seed coat, out of the soil (fig. 1, *g*). Usually within about 2 days' time the

FIGURE 1.—Germinating seeds in various stages of development after planting, the time indicated having elapsed: *a*, 12 hours; *b*, 2 days; *c*, 3 days; *d*, 4 days; *e*, 5 days; *f*, 6 days; *g*, 7 days; *h*, 9 days; *i*, 12 days.

hypocotyl straightens and the seedling assumes a vertical position. The indehiscent seed coat almost always persists, partly enclosing the cotyledons for about 2 or 3 days, after which it drops (fig. 1, *h*, *i*). The entire process as conducted in the greenhouse at a temperature of 85° to 100° F. requires from 5 to 10 days but is progressively lengthened as the temperature is lowered (*3*).

ANATOMY OF THE YOUNG PRIMARY ROOT

The primary xylem of the root of *Capsicum frutescens* is diarch, no exceptions to this being found. As is typical of roots the primary xylem and phloem have the usual radial arrangement (pl. 1). There are from 4 to 6 cells in each of the 2 protoxylem groups and these are separated by from 20 to 30 metaxylem elements. The primary xylem elements are spirally thickened in both the root and hypocotyl. The thickenings range from loose spirals to the typical pitted vessels.

The primary phloem is composed of sieve tubes, companion cells, and phloem parenchyma cells. Immediately outside the primary phloem and abutting the protoxylem is a continuous layer of irregularly shaped cells constituting the pericycle. The endodermis is present immediately outside the pericycle and consists of one layer of cells. However, in some sections it was rather difficult to determine.

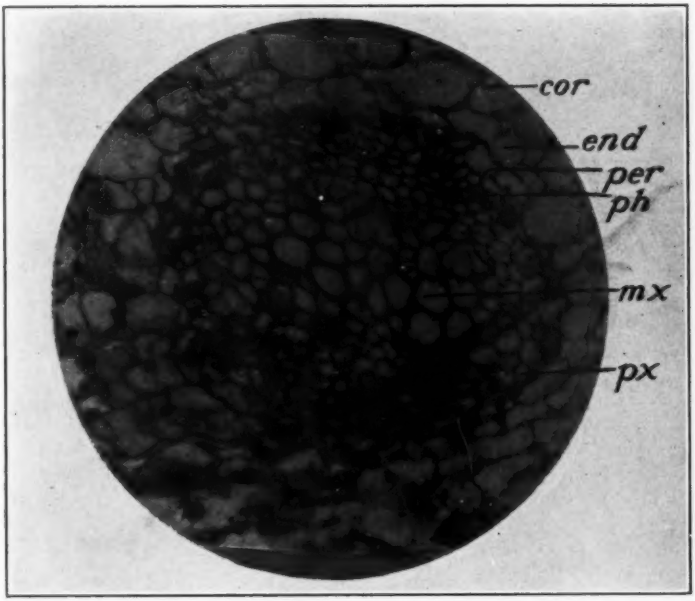
The cortex is composed for from 8 to 10 layers of loosely arranged parenchyma cells and numerous rather large intercellular spaces. This tissue makes up approximately 80 percent of the entire primary root. The epidermis is one layer of cells in thickness. No fibers are present in the root previous to the time secondary thickening takes place. Lateral roots originate from the pericycle in regions intermediate between the protoxylem points and primary phloem groups.

ROOT-STEM TRANSITION

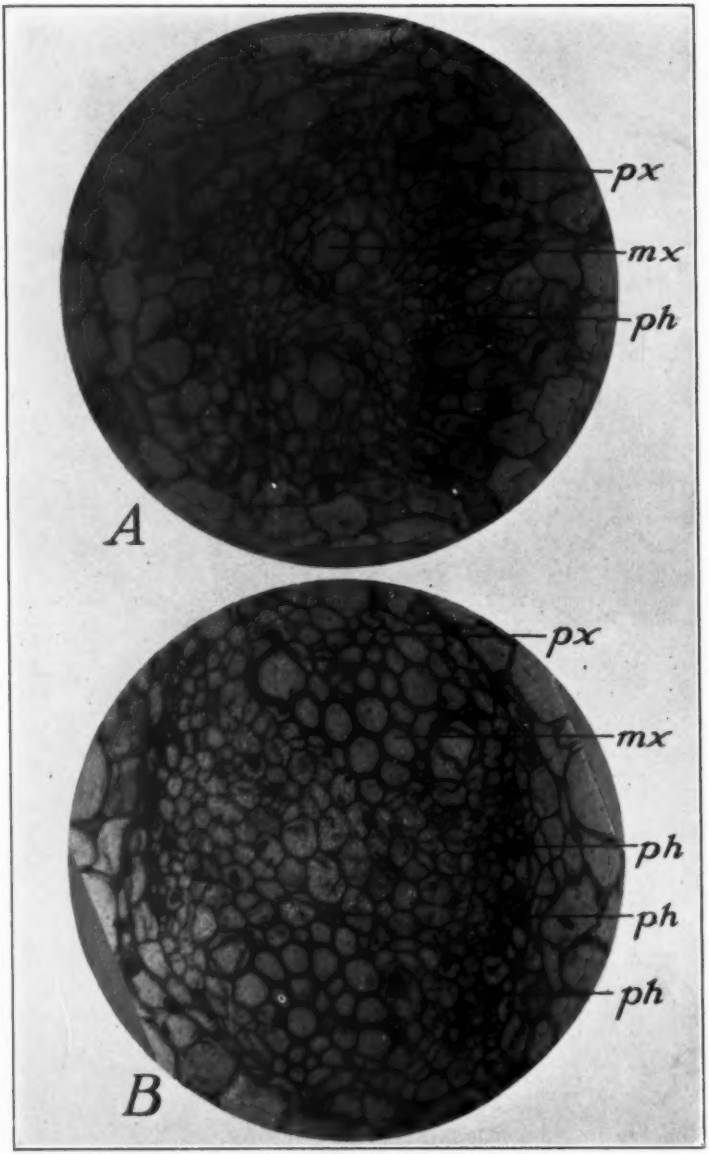
The first indication of a change from the exarch to the endarch condition occurs in the lowermost portion of the hypocotyl. This change is the breaking-up of the diarch xylem plate with the pith becoming evident (pl. 2, *A*), and the beginning of a bifurcation of these two formed xylem strands (pl. 2, *B*). In some cases the bifurcation is evident before the xylem plate separates into two distinct parts. Simultaneous with or immediately after this change the two phloem groups split up into three strands each (pl. 2, *B*). In the lower portion of the hypocotyl, about 2.5 cm. below the cotyledons, the central group of phloem cells, on either side, differentiates inward to form the internal phloem (pl. 3, *A*). These inward differentiating phloem groups divide to form five to eight internal phloem groups. The external phloem groups divide into several strands. The strand nearest the protoxylem points soon come to lie opposite the four metaxylem groups. This differentiation of the phloem groups is accompanied by further bifurcation of the xylem groups (pl. 3, *B*).

The change from root to stem is very gradual over most of the length of the hypocotyl. No decided change takes place in root-stem transition from the lower portion of the hypocotyl, until a point from 2 to 5 mm below the cotyledons is reached. At this point the protoxylem points become separated from the two metaxylem groups by parenchymatous cells. At a level just below the divergence of the cotyledons internal phloem groups assume a position on the inner face and external phloem groups on the outer face of each of the two metaxylem strands, lateral to the two protoxylem points, thus forming four bicollateral bundles (pl. 4, *A*). At this point there is also a differentiation of primary xylem on each of the inner lateral sides of each of these four bundles. These primary xylem strands constitute the internodal foliar traces. Outer phloem groups do not appear directly opposite the original position of the protoxylem points until a point is reached 2 to 10 mm out in the midrib of the cotyledon.

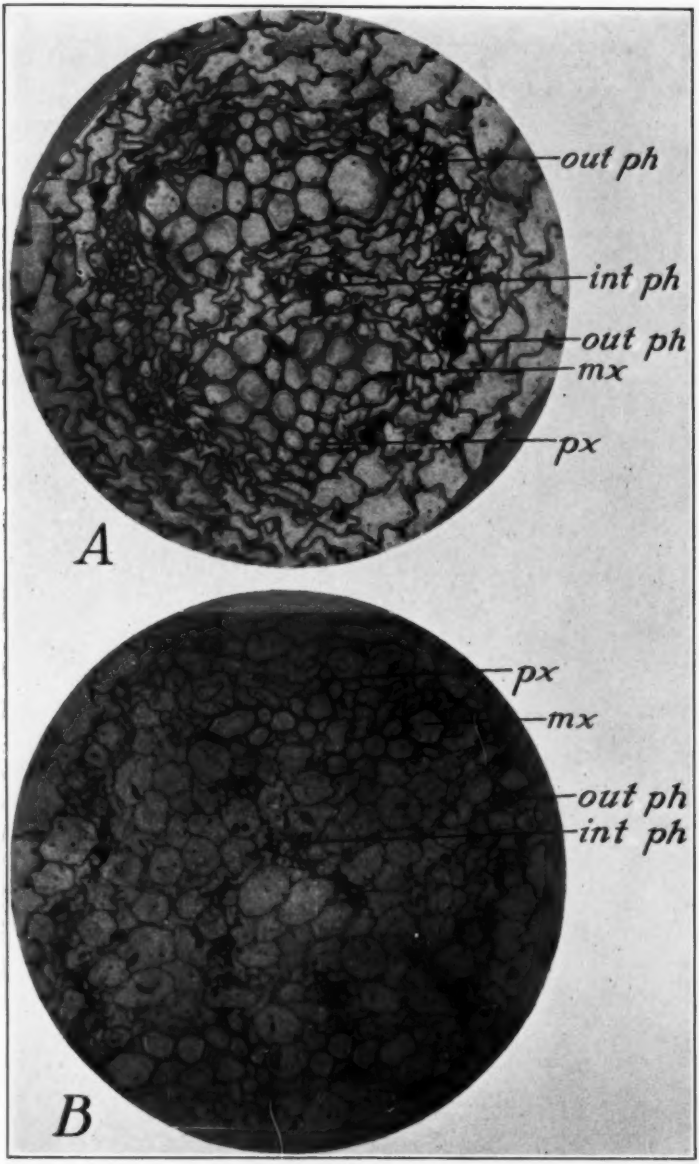
About 2 mm below the cotyledonary plate there is a gradual separation of the two bundles formed by the breaking up of the original diarch xylem plate. One of these double units consisting of one protoxylem point and its two lateral metaxylem groups, together with the internal and outer phloem groups, becomes the vascular trace of one of the cotyledons and the second that of the other.



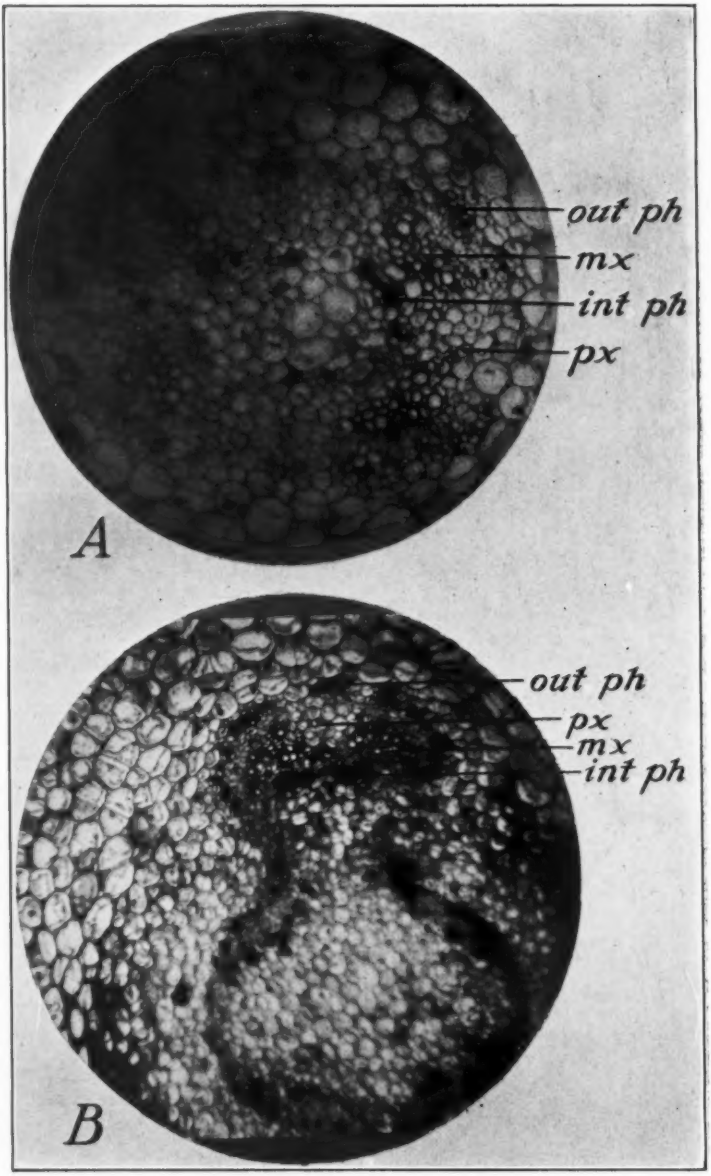
Transverse section of young primary root. *cor*, Cortex; *end*, endodermis; *mx*, metaxylem; *per*, pericycle; *ph*, phloem; *px*, protoxylem. × 225.



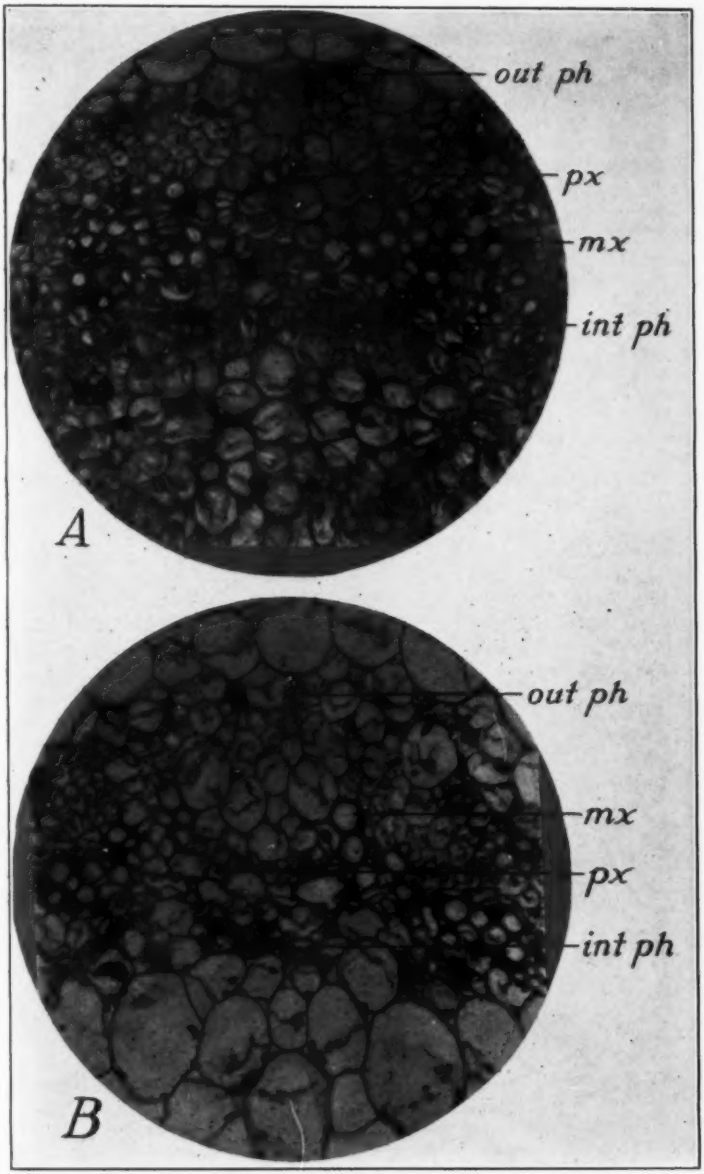
A, Transverse section of lower region of hypocotyl showing xylem plate divided into two parts; *B*, transverse section of hypocotyl at a higher level than in *A*, showing bifurcation of metaxylem and division of phloem into three strands. *mx*, Metaxylem; *ph*, phloem; *px*, protoxylem. × 225.



A, Transverse section of hypocotyl at a higher level than in plate 2, *B*, showing differentiation of central phloem strand to form internal phloem and further bifurcation of metaxylem; *B*, transverse section of upper portion of hypocotyl showing complete bifurcation of metaxylem and numerous outer and internal phloem strands. *mx*, Metaxylem; *int ph*, internal phloem; *out ph*, outer phloem; *px*, protoxylem. × 225.



A, Transverse section of hypocotyl just below cotyledonary plate, showing separation of double bundles as traces to cotyledons and position of phloem strands; *B*, transverse section near cotyledonary plate, showing inward differentiation of protoxylem in cotyledonary trace and the position of the outer and internal phloem strands. *int ph*, Internal phloem; *mx*, metaxylem; *out ph*, outer phloem; *px*, protoxylem. *A*, × 150; *B*, × 100.



A, Higher magnification of cotyledonary trace shown in plate 4, *B*. Section through petiole of cotyledon 1.5 mm above cotyledonary plate. Note inward differentiation of protoxylem and position of outer and internal phloem strands. *B*, Transverse section through midrib of cotyledon 3 or 4 mm above cotyledonary plate showing complete centripetal differentiation of protoxylem. *int ph*, Internal phloem; *mx*, metaxylem; *out ph*, outer phloem; *px*, protoxylem. × 225.

The protoxylem points begin to change their position just before the complete divergence of the cotyledons (pl. 4, *B*). This differentiation is centripetal and at the successively higher levels in the midrib of the cotyledons the protoxylem elements gradually assume a position on the inner tangential face of the two metaxylem groups. The two metaxylem groups in the trace of each cotyledon swing toward the original position of the protoxylem points. In most seedlings studied these two groups never quite meet. Likewise, outer phloem groups are differentiated toward the original position of the protoxylem points and become located opposite that position (pl. 5, *A*). These phloem and xylem groups, together with the internal phloem strands, eventually constitute what is essentially a single, bicollateral, endarch bundle. The transition from root to stem is complete at a point about 5 mm out in the midrib of the cotyledon (pl. 5, *B*).

DISCUSSION

One of the primary objects of this investigation was to note the similarity, if any, between the changes that take place in the root-stem transition of *Capsicum frutescens* Linn., and that of several other members of the Solanaceae family hitherto studied.

The method of root-stem transition is relatively uniform for the reported plants of the family as is shown by the work of King, Clark, and Thiel for *Lycopersicum esculentum*, of Thiel for *Solanum melongena*, *S. tuberosum*, *Datura tatula*, *Physalis verginiana*, and *S. pseudocapsicum*. All these studies are in general agreement with the findings of this investigation for *Capsicum frutescens*.

The results of this work, as well as of that mentioned above, are in full agreement with those of Artschwager for *Solanum tuberosum* as far as the phloem is concerned, but in disagreement with his findings concerning the xylem. At no time was it noted that two primary xylem groups swung outward with one following a left, the other a right curve. Instead of the two primary xylem groups following different curves there was a complete bifurcation of the metaxylem.

SUMMARY

The method of root-stem transition in *Capsicum frutescens* is essentially the same as that in several other previously reported members of the Solanaceae family.

The epidermis of the primary root is 1 layer of cells in thickness; the cortex is composed of from 8 to 10 layers of loosely arranged parenchyma cells and numerous rather large intercellular spaces. This tissue makes up approximately 80 percent of the entire young root. The endodermis and pericycle each consist of one layer of cells, the latter giving rise to lateral roots.

The stele of the primary root is a diarch, radial protostele.

The first indication of a change from the exarch to the endarch condition occurs in the lowermost portion of the hypocotyl. This change is the breaking up of the diarch xylem plate and the two primary phloem groups with the pith becoming evident, and the beginning of a bifurcation of the two formed xylem stands. Simultaneous with or immediately after this change the two phloem groups split up into three strands each.

In the lower portion of the hypocotyl, about 2.5 cm below the cotyledons, the central group of phloem cells on either side become differentiated inward to form the internal phloem.

At a level just below the divergence of the cotyledons internal phloem groups assume a position on the inner face and external phloem groups on the outer face of each of the metaxylem strands.

About 2 mm below the cotyledonary plate there is a gradual separation of the two bundles formed by the breaking up of the original diarch xylem plate. One of these double units, consisting of one protoxylem point and its two lateral metaxylem groups together with the internal and external phloem groups, becomes the vascular trace of one of the cotyledons, and the second unit, that of the other.

Inward differentiation of the protoxylem with an orientation of the metaxylem and phloem groups eventually leads to the establishment of the bicollateral condition.

The complete transition from root to stem is not attained in the hypocotyl but at a point about 5 mm out in the midrib of the cotyledon.

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MEASUREMENT OF THE VISCOSITY OF EGGS BY THE USE OF A TORSION PENDULUM[1]

By J. V. ATANASOFF, *associate professor of mathematics and physics, department of physics, Iowa State College,* and H. L. WILCKE, *professor and head, poultry husbandry subsection, Iowa Agricultural Experiment Station*

EARLIER METHOD

During the spring of 1933 the problem of determining the viscosity of an egg from external data arose in connection with a study of other properties of eggs. Dr. Lyle Goodhue, formerly of the chemistry department of Iowa State College, suggested the possibility of using the damping of a torsion pendulum on which the egg was placed as a measure of this viscosity, and he designed apparatus to make these measurements. At his suggestion, the contents of eggs within shells of various sizes were removed and replaced by glycerol solutions of known viscosities. These eggs were then used to standardize the apparatus, the idea being that in this indirect way the viscosity of the egg contents could be measured. There are, however, three important difficulties with the method so far developed.

(1) An egg is not homogeneous in its interior, but consists of a central core structure composed of the yolk more or less firmly attached to the stiff albumen. This core structure floats in the highly fluid thin albumen which forms a lubricating layer inside the shell, so that measurements made by reference to a homogeneous model (i. e., the eggshell filled with glycerol solution) cannot be expected to be directly significant.

(2) The method employed is not complete enough to allow the apparatus to be easily standardized. No check was used to detect changes in the characteristics of the apparatus, and its physical properties were not given so that new apparatus could be produced which would yield the same results.

(3) Perhaps the most serious difficulty, and the one which initiated the present collaboration, is an ambiguity in the interpretation of the results of measurement with a torsion pendulum. If an eggshell is filled with a very thin liquid and the shell is oscillated in the torsion pendulum the inner portions of the liquid stand still and there is little damping of the pendulum. On the other hand, if the eggshell is filled with a very viscous liquid the egg will tend to turn as a solid body, and again there will be little damping of the pendulum. For each amount of damping there will be different viscosities which the contents of the egg can have. This result obtained by physical intuition is readily verified by experiment. Figure 1 shows the results of some experiments with an eggshell filled with glycerol solution. The coefficient of damping, K, of the torsion pendulum (calculated from experimental data in a way to be described later) is plotted against the Saybolt viscosity of the glycerol solutions. It is evident that for a given value of the coefficient K, there are two possible viscosities.

[1] Received for publication Oct. 16, 1936; issued June, 1937. Journal Paper No. J-308 of the Iowa Agricultural Experiment Station. Project No. 50.

The foregoing difficulties have caused the writers to change the method somewhat. In the first place, they have assumed a dynamical model of an egg which consists of a solid central portion to represent the yolk and firm albumen surrounded by a viscous fluid to represent the thin albumen.[2]

The second and third difficulties have been resolved by the use of the methods of mathematical physics. These results illustrate the use of mathematical-physical methods in biological problems as contrasted with the application of statistical mathematics. While the great utility of the statistical methods is generally recognized, the use of mathematics in its physical applications does not seem so well known in the biological field.

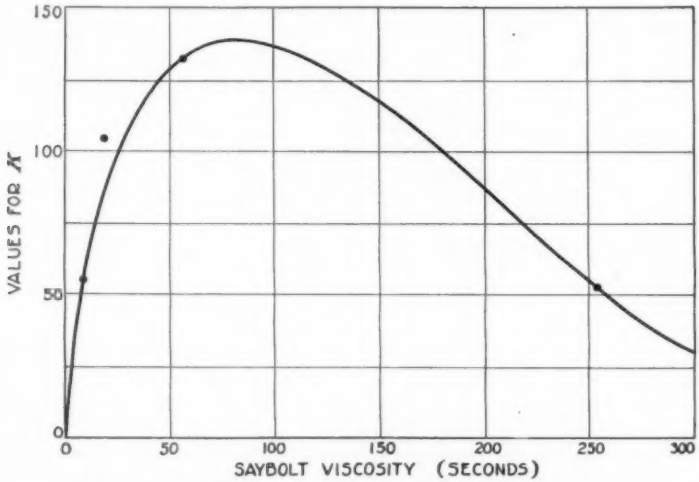

FIGURE 1.—Variation of K with Saybolt viscosity of eggshells containing glycerin.

EXPERIMENTAL METHOD

Figure 2 is a photograph of the apparatus used. A light metal frame, f, supports a horizontal hard rubber ring, r, in which the egg, e, may be placed with its small end downward; b is a brass rod upon which the weights, w, w', slide to vary the moment of inertia of the frame. These weights are retained in a given position by setscrews. The frame is suspended between fixed supports by steel piano wire. A small mirror, m, attached to the upper end of the frame reflects a spot of light from the lamp house, l, onto a screen and enables one to follow the oscillations of the system.

When the system is slightly twisted and released it oscillates with an angular motion with the vertical wire supports as an axis. The period and frequency depend upon the moment of inertia of the system and the torsion constant of the wire supports. The period is deter-

[2] The existence of thin albumen enmeshed within the firm albumen has no significance in the present problem. Moreover, it is not necessary to take the fluidity of yolk into consideration, as a sufficiently accurate model can be obtained without this refinement.

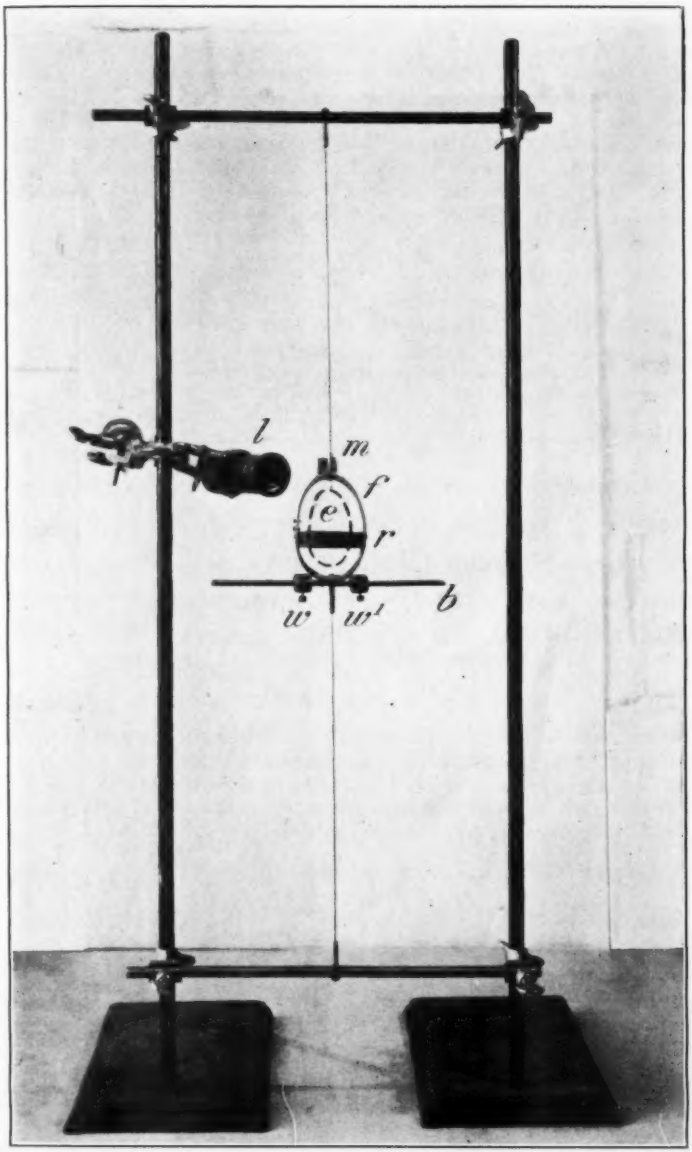

FIGURE 2.—Apparatus used in determining egg viscosity: *f*, Light metal frame; *r*, horizontal hard-rubber ring; *e*, the egg; *b*, brass rod; *w*, *w'*, weights; *m*, small mirror; *l* lamp.

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mined by timing some convenient number of swings. It is, of course, observed that the angular amplitude decreases with time, and this decrease of amplitude is called the damping of the system. The damping is measured by taking either the number of swings or the time necessary for the angular amplitude to decrease from a certain value, θ', to a smaller value, θ'', as indicated by marks on the screen. The counting of swings has proved a more satisfactory method as it allows a mental interpolation if, as is usual, the extreme position of no single swing falls on the mark. Different observers doing this mental interpolation can generally interpolate at both marks and agree to within one- or two-tenths of a swing.

THEORETICAL BASIS

Let

θ=Angular position of frame from rest position.
I=Moment of inertia of frame.
μ=Torsion constant of wire.
c=Coefficient of damping resistance.
t=Time.

The differential equation of motion of the frame is $I\frac{d^2\theta}{dt^2}+c\frac{cd\theta}{dt}+\mu\theta=0$.

It is convenient to divide this equation by I and use the new letters $l=\frac{c}{2I}$, $m^2=\frac{\mu}{I}$. In terms of these abbreviations the solution is $\theta=ae^{-lt}\cos(\omega t-\beta)$ in which $\omega=\sqrt{m^2-l^2}$, and a and β are arbitrary constants. The frequency, $f=\frac{\omega}{2\pi}$, and period, $P=\frac{2\pi}{\omega}$, are more often used in practical work than the angular frequency ω. The logarithmic damping $\delta=lP$ is easily seen to be the natural logarithm of the ratio of two successive amplitudes. If δ and I are small $\omega=\frac{2\pi}{P}=m=\sqrt{\frac{\mu}{I}}$. Now we shall adopt the convention that quantities measured with the empty frame go unmarked, that quantities measured with the bar of known moment of inertia, I', in the frame are marked with a bar above them while the presence of an egg will be denoted by a subscript on the quantities under consideration. Obviously $\bar{\mu}=\mu_1=\mu$. So from the last equation $\frac{2\pi}{\bar{P}}=\sqrt{\frac{\mu}{\bar{I}}}$, while of course $\frac{2\pi}{P}=\sqrt{\frac{\mu}{I}}$. Hence, we have $\frac{\bar{P}^2}{P^2}=\frac{\bar{I}}{I}$. But $\bar{I}=I+I'$ so $I=I'\frac{P^2}{\bar{P}^2-P^2}$. This relation is used to determine I.

An egg is represented by a central core structure of moment of inertia, J, surrounded by a viscous fluid which yields a torque T proportional to the difference of the angular velocities of the core $\frac{d\phi}{dt}$ and the shell $\frac{d\theta}{dt}$.

$$T=k\left(\frac{d\phi}{dt}-\frac{d\theta}{dt}\right)$$

When the egg is placed in the frame the resulting differential equations are easy to formulate and possible of solution, but this rigorous solution is intricate enough in form to be hard to use. A method of

approximation is indicated by the fact that the moment of inertia of the egg is always small compared with that of the frame structure. So the reaction of the egg on the frame does not change its motion much except to increase its damping.

At first we assume the motion of the shell is purely sinusoidal, that is we may write $\theta = a \sin \omega t$. Now by Newton's law for rotary motion, $k(\theta - \phi) = J\ddot{\phi}$, in which J is the moment of inertia of the core of the egg and the dots denote differentiation with respect to time. Thus, $\dot{\phi} = \frac{d\phi}{dt}$ and $\ddot{\phi} = \frac{d^2\phi}{dt^2}$. Since θ is known we may easily solve this differential equation. The particular integral $\phi = \frac{ak^2}{k^2 + J^2\omega^2} \sin \omega t - \frac{akJ\omega}{k^2 + J^2\omega^2} \cos \omega t$ describes the sustained motion of the core structure which is needed for the present problem.

We may now calculate the work done by the shell on the contents of the egg during one cycle. It is $W = \int T d\theta = \frac{\pi k a^2 J^2 \omega^3}{k^2 + J^2\omega^2}$. If the same method is applied to the normal damping torques, $T = c\dot{\theta}$, the work done per cycle is found to be $W = \int T d\theta = c\pi a^2 \omega$. So, if one writes $I\ddot{\theta} + c_1\dot{\theta} + \mu\theta = 0$ in which $c_1 = c + K$, $K = \frac{kJ^2\omega^2}{k^2 + J^2\omega^2}$, one will have a differential equation furnishing nearly the correct solution for θ, for it will have the correct damping, and the egg is too small to affect the motion in any other way. We have chosen K as the measure of total egg viscosity.[3] It is easily calculated, in fact we have, $\delta = lP = \frac{c}{2I}P$. Likewise, $\delta_1 = l_1 P_1 = \frac{c_1 P_1}{2I}$. But the effect of an egg on P is too small to be important so that we may write $\delta_1 = \frac{c_1 P}{2I}$. So $K = c_1 - c = \frac{2I}{P}(\delta_1 - \delta)$. As before stated, δ is found by counting the number of swings needed for the pendulum to damp between two angular amplitudes θ' and θ''. The formula is $\delta = \frac{1}{n} \log \frac{\theta'}{\theta''}$. So we finally find $K = \frac{2I}{P}\left(\frac{1}{n_1} - \frac{1}{n}\right) \log_e \frac{\theta'}{\theta''}$. The angular amplitudes θ' and θ'' are determined by marks on the screen on which the spot of light moves. In this way $\log_e \frac{\theta'}{\theta''}$ becomes a constant of the apparatus.

The formula for K, $K = \frac{kJ^2\omega^2}{k^2 + J^2\omega^2}$, is worth further study. If k be plotted against K we have a curve of the form shown in figure 3. The fact that there are two values of k for a given K and hence for a given damping of the torsion pendulum is clearly shown.

A plot of the variation of K with $\omega = 2\pi f$ is also instructive as it represents a relation that one may actually obtain in the measurement of a single egg. The relation of figure 3 cannot be thus realized, for the k of a given egg is a fixed quantity. However, before plotting the

[3] However, see the remarks about the relation of K and k on page 14.

equation we write it in the form $\frac{K}{k}=\frac{(J\omega/k)^2}{1+(J\omega/k)^2}$, that is, $Z=\frac{X^2}{1+X^2}$, $Z=\frac{K}{k}$, $X=\frac{J\omega}{k}$. This transformation of variables reduces the graph to

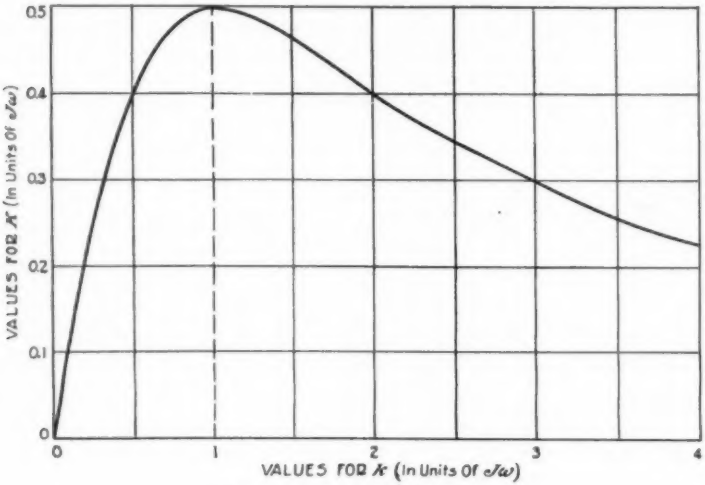

FIGURE 3.—Result of plotting k against K.

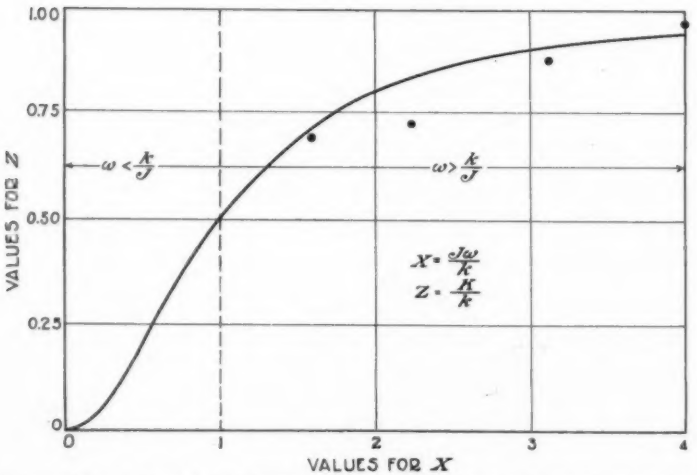

FIGURE 4.—Values for X and Z plotted after transformation of variables reducing the graph to a form applicable to any J and k.

a form applicable to any J and k. A glance at this graph (fig. 4) shows that as X increase Z approaches unity; that is, K approaches k.

DISCUSSION OF EXPERIMENTAL RESULTS

In a typical experimental run the damping effects of each of 12 eggs were measured at four different frequencies. The corresponding values of K were calculated and are presented in table 1.

TABLE 1.—*Variation of K with the frequency f at which it is measured*

f	ω	Data for egg no.—											
		1	2	3	4	5	6	7	8	9	10	11	12
0.675	4.25	131	65	74	72	175	67	129	71	111	63	82	73
1.01	6.33	104	70	72	88	212	73	124	82	82	61	71	73
1.43	8.97	115	85	86	97	190	88	122	97	97	72	81	81
1.83	11.5	113	93	93	97	158	92	128	106	105	83	85	87

It is rather clear that, in a general way, the variation of K with ω for most eggs fits the theoretical result indicated in figure 4. The points marked on the graph indicate the experimental results for the second egg when $J=34$ g, cm^2 and $k=97$. They agree well with the theoretical curve. The most troublesome feature of the experimental results is a tendency for K either to decrease or not to increase fast enough for low values of ω. This is strongly portrayed by the tabular results for eggs 1 and 9, and is present in eggs 3, 7, 10, and 11. This effect cannot be explained in terms of the model used but probably is caused by an initial breaking down of the egg structure as the experiment proceeds, causing the value of K to decrease as ω increases. As an example, the K value of egg no. 2863 was 347 when the determination was made at a frequency of 1.83 and at the amplitude given below. However, when this egg was severely shaken, and a second determination was made immediately after shaking, the K value had decreased to 136, and when this was repeated the K value dropped to 125. This indicates the desirability of treating the egg gently in the measuring process in order to avoid errors due to injury of the egg structure. This can be accomplished by conducting the measurements at small amplitude (i. e., small θ').

The writers used the values $\theta'=33°$ and $\theta''=23.7°$, but there is no objection to using smaller values, which would, of course, decrease the internal forces in the egg and prevent a partial disintegration of its structure.

The quantity K reaches its maximum value when $k=J\omega$ (fig. 3). As long as ω is maintained either above or below the value $\frac{k}{J}$ there is no question of an ambiguity in the value of k for a given K. For a reason to be mentioned presently the writers have seen fit to make the measurements in the range $\omega>\frac{k}{J}$, that is $X=\frac{J\omega}{k}>1$ (fig. 4). Except in these theoretical investigations of the method all measurements were made at the highest frequency, which makes X well above unity and in addition makes Z near unity, or K near k. This is the advantage of making the observations at relatively high frequency: Although K is given as the measure of egg viscosity, it is measured at a frequency which makes it nearly equal to the value of

k. This value of k will therefore depend only slightly on the exact frequency used. The other effects due to the measuring apparatus have of course been entirely eliminated.

In order to determine the relationship between the K values as determined by the torsion pendulum and commercial grading, comparisons were made between the K values and the two candling factors, yolk shadow and yolk movement, for a large number of eggs. For this purpose, all of the eggs from a number of pens were placed in a controlled temperature cabinet in the evening of the day on which they were laid, and the K values were determined the following day. Immediately after the K values were obtained the eggs were candled[4] and divided into five classes of yolk shadow and yolk movement ranging from light to dark for the shadow, and slow to fast for yolk movement.

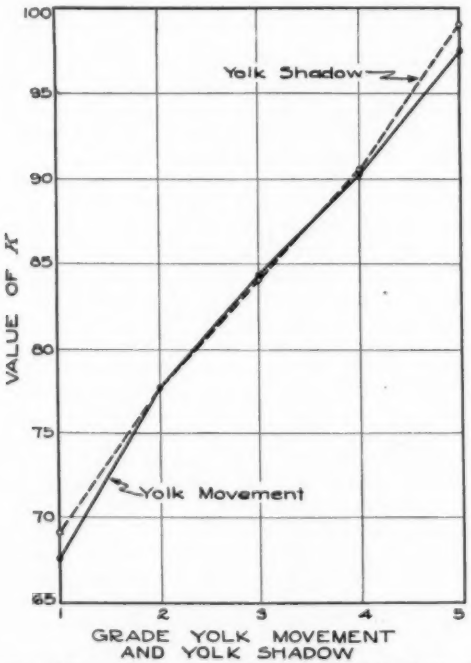

FIGURE 5.—Regressions of K on yolk movement and yolk shadow plotted for the five classifications of each of these two factors.

A total of 3,890 eggs was used in this study. The K values for the eggs were corrected for the period and moment of inertia of the torsion pendulum, and the number of swings to damp the empty pendulum. This was accomplished by means of the formula $K=\frac{2I(.329)}{P}\left(\frac{1}{N_1}-\frac{1}{N}\right)$, which is derived above. The regressions of K on yolk movement and yolk shadow were plotted for the five classifications for each of these two factors. These regression lines are shown in figure 5. There is a

[4] The classification of eggs into grades of yolk movement and yolk shadow was made by Dr. F. D. Perry, of Armour & Co.

very close relationship between yolk shadow and K value when the K value is not corrected for the weight of the egg; that is, in general, the eggs which show the most rapid yolk movement before the candle also exert the least damping effect upon the pendulum, and consequently yield the lower values for K. Similarly, those eggs which are classified as having a very distinct yolk shadow generally have the lower K values. It must be clearly understood, however, that these regression lines are only trend lines, and that individual eggs may vary considerably. This point is more clearly illustrated by the correlation coefficients for K and each of these candling factors. The correlation coefficient for K and yolk movement was found to be 0.2388 for this group of 3,890 eggs. For K and yolk shadow, the correlation coefficient was 0.2286. These correlation coefficients, while highly significant, are rather low, which may be accounted for by the large variation between individual eggs. Therefore, it may be concluded that the torsion pendulum method yields results which are in general agreement with results obtained by grading eggs before the candle; that is, that eggs which have a low total viscosity index are, in general, of poor quality as judged by yolk movement and yolk shadow. When individual eggs are studied, however, there is more variation in the results than would be desirable from an experimental viewpoint.

LOCALIZATION OF PENTOSANS IN THE RESIN GLANDS OF THE COTTON EMBRYO[1]

By R. G. REEVES, *professor of biology, Agricultural and Mechanical College of Texas*, and J. O. BEASLEY, *assistant agronomist, Texas Agricultural Experiment Station*

INTRODUCTION

In a previous publication, Reeves and Valle (*4*)[2] gave the probable localization of cellulose, lignocellulose, pentosans, sugars, proteins, and oil in the cotton embryo. All of these components had been reported by other investigators as being present, but their localization had not been investigated. The localization of most of these substances, except pentosans, seemed conclusive from the results of the tests. The only organ or tissue of the embryo which gave any indication of pentosans was the resin glands, but the reactions obtained in these were not typical for pentosans. Pentosans are localized in cell walls in many structures, but seldom, if ever, in the cell walls of embryos. Besides, the cell walls of the cotton embryo were found to be composed chiefly of cellulose, and no indication of pentosans was found in them. Since the appearance of the publication mentioned above, further studies have been conducted in an effort to determine the localization of pentosans in the embryo, and the results are reported herein.

MATERIAL AND METHODS

Resin glands of both the leaves (exposed to light) and of the seed (unexposed) were included in the investigation in order to prevent confusion and the apparent contradiction that could result from any differences that might exist between the two; however, those of the seed were of primary interest. As it was found that treating the glands with certain acids alone produced marked color reactions, and as the tests used were not considered specific for pentosans, it was thought advisable to treat the glands with various concentrations of several acids. The results of these tests are useful in evaluating the usual pentosan reactions when applied to the cottonseed, because the pentosan tests, when used microchemically, depend upon color changes brought about by treating pentosans with phenolic compounds, such as phloroglucinol and hydrochloric acid. By treating the glands with these acids, it was possible to determine whether the color reactions obtained when testing for the probable constituents of the resin glands could have been caused by acids alone.

The colors resulting from the tests made in this work were identified according to Ridgway (*5*). In a few instances the colors obtained were not exact duplicates of any given by Ridgway, and in such cases the colors that most nearly matched were recorded. However, in all such instances the differences were slight. The most satisfactory record of the colors was found to be a statement of the fundamental

[1] Received for publication Nov. 5, 1936; issued June 1937. Contribution from the Division of Agronomy, Texas Agricultural Experiment Station, Technical Paper No. 371.

[2] Reference is made by number (italic) to Literature Cited, p. 718.

color, plate number, color or hue number, and tone, respectively, as given by Ridgway.

Except when statements to the contrary are made, the results were observed at 100 × magnification in white transmitted light. The preparations were examined while the reagents were being applied or immediately thereafter, and were kept under observation until all observable reactions ceased.

The correctness of the term "resin glands" when applied to the structures discussed here is doubtful and will remain so until the fundamental nature of the structures is better understood. However, this use of the term is well established and the writers will make no attempt to change it at this time.

RESULTS OF TREATING GLANDS OF COTTON LEAVES WITH ACIDS AND BASES

Untreated glands in the leaves were very dark violet (this shade of violet is not given by Ridgway); in fact, the color was so intense that it appeared almost black. Immediate observations on the glands that were ruptured when the leaf was sectioned showed the gland contents to be red violet (11, 61, k). Whether this change upon sectioning was merely the result of diluting the original color, or whether it was a fundamental change, was difficult to determine. When the glands were simply punctured with a needle the same change occurred as when they were cut and a simultaneous spreading of the gland contents took place. The best interpretation seemed to be that the change resulted merely from a dilution of the original color, which was brought about by a spreading of the gland contents.

In order to determine more clearly whether sulphuric acid (table 1) would produce the red color or whether it was produced by the spreading of the gland contents, some whole normal leaves were placed in dishes and covered with sulphuric acid of various concentrations. After about 20 minutes, every gland in all concentrations was distinctly red (of various shades and tints), and had partially dissolved. The acid was then replaced with a solution of ammonia which turned the glands blue. Many repetitions of this test gave the same results.

RESULTS OF TREATING GLANDS OF COTTON EMBRYOS WITH ACIDS AND BASES

Sulphuric acid alone, either of commercial strength or diluted to 66 percent, produced red colors in the unexposed glands and caused the contents of these glands to emulsify rapidly (table 2). Yellow, and later brown, colorations occurred in the tissues. The mixing of red and yellow colors thus produced sometimes gave an orange color. A red color was rarely produced in the tissues. With concentrations of acid as low as 50 percent the glands emulsified with a yellow color (16, 21′, i).

TABLE 1.—*Results of microchemical tests on leaves of upland cotton*

Reagent used [1]	Reaction in glands		Color [2] in tissues
	Color [2]	Solubility	
Water	Red (12, 71, b)	Dissolving when pressed from glands.	Green (6, 33, k).
Sulphuric acid, specific gravity 1.84, undiluted.	Red (13, 3′, —)	Dissolving	Green yellow (5, 27, i).
Sulphuric acid, diluted to 83 percent.	Red (13, 5′, b)	do	Green (31, 27″, k).
Sulphuric acid, diluted to 66 percent.	Red (13, 3′, d)	do	Do.
Sulphuric acid, diluted to 50 percent.	do	do	Green (6, 33, k).
Nitric acid, specific gravity 1.41, undiluted.	Red (27, 5″, —)	do	Yellow green (4, 23, k).
Nitric acid, diluted to 83 percent	Red (27, 3″, —)	do	Do.
Nitric acid, diluted to 66 percent	do	Slowly dissolving	Do.
Nitric acid, diluted to 50 percent	do [3]	Dissolving	Green (5, 27, k).
Hydrochloric acid, specific gravity 1.18, undiluted.	Red (13, 5′, d)	do	Green (31, 29″, i).
Hydrochloric acid, diluted to 50 percent.	Red (12, 71, d)	do	Green (5, 25, k).
Acetic acid, 99 percent, undiluted.	Red (26, 71′, b)	do	Yellow (16, 21′, i).
Acetic acid, diluted to 50 percent	do	do	Do.
Ammonium hydroxide, specific gravity 0.9, undiluted.	Blue (22, 51*, k)	Partially dissolving	Green (6, 31, k).
Ammonium hydroxide, diluted to 83 percent.	Blue (22, 39*, k)	do	Do.
Ammonium hydroxide, diluted to 63 percent.	Blue (22, 49*, k)	Dissolving	Do.
Ammonium hydroxide, diluted to 50 percent.	do	do	Do.
Potassium hydroxide, 10 percent	Green (18, 35′, b)	do	Green (17, 29′, —).
Potassium hydroxide, 5 percent	do	do	Do.
Phloroglucinol, alcoholic solution.	Red (12, 71, b)	do	Chlorophyll partially dissolves to green (17, 29′, —).
Alcohol and hydrochloric acid	Red (26, 71′, b)	do	Green (5, 27, k).
Phloroglucinol and 50 percent hydrochloric acid.	do	do	Green (6, 31, k).
Orcinol and hydrochloric acid (Mathews (*2a*)).	Red (12, 71, b)	do	Green.
Naphthoresorcinol and 50 percent hydrochloric acid.	do	do	Do.

[1] The concentrations of all of the acids and of the ammonium hydroxide used in this work were measured volumetrically.

[2] In parentheses are given first the plate number, second the color or hue number, and third the tone (Ridgeway (*5*)).

[3] Several minutes required for change.

Although the color reaction obtained in the glands by treating with sulphuric acid is well known and has been attributed by Marchlewski (*2*) to the presence of gassypol, no report of this reaction in the other parts of the cotton embryo has been found in the literature.

When hydrochloric acid was applied to the embryo and the preparation was heated, a red (12, 69, f) color sometimes appeared throughout the tissues but not in the glands. The failure of the glands to give a red color was of regular occurrence irrespective of the amount of heat used or of the concentration of the acid. Although the contents of the glands often streamed out, their only change in color was to green or yellow. More than a hundred tests were made in which different concentrations of acid ranging from 25 to 100 percent of commercial strength were used, but none gave any type of red color. This shows that hydrochloric acid does not affect the glands as does sulphuric acid. The failure of hydrochloric acid alone to affect the glands is a noteworthy observation, for if it, like

sulphuric acid, had given a red coloration, the phloroglucinol-hydrochloric acid reaction for pentosans would be unsatisfactory. When alcohol, which is the usual solvent of phloroglucinol, and hydrochloric acid were applied to the glands, no reaction was observed that resembled the phloroglucinol-hydrochloric acid test for pentosans.

TABLE 2.—*Results of microchemical tests on embryos of upland cotton*

Reagent used	Reaction in gland		Color [1] in tissue
	Color [1]	Solubility	
No reagent (dry)	Yellow (30, 23″, —)		No change.
Water	Green (4, 23, m)	Rapidly emulsifying	Green (4, 21, m).
Sulphuric acid, specific gravity 1.84, undiluted.	Red (13, 3′, —)	Dissolving	Streaks of yellow (5, 27, d) and red (12, 69, f); decomposing.
Sulphuric acid, diluted to 83 percent.	Red (1, 3, —)	do	Do.
Sulphuric acid, diluted to 66 percent.	Red (1, 3, b)	do	Streaks of yellow (5, 27, d); slowly decomposing.
Sulphuric acid, diluted to 50 percent.	Yellow (16, 21′, i)	do	
Nitric acid, specific gravity 1.41, undiluted.	do	do	Yellow (30, 23″, f); very light.
Nitric acid, diluted to 83 percent.	Yellow (5, 25, i)	do	Yellow (30, 23″, f).
Nitric acid, diluted to 66 percent.	Green (30, 19″, k)	Dissolving or emulsifying.	No change.
Nitric acid, diluted to 50 percent.	do	Emulsifying	Do.
Hydrochloric acid, specific gravity 1.18, undiluted.	Yellow (16, 21′, i)	do	Colorless; after boiling, red (12, 69, f).
Hydrochloric acid, diluted to 50 percent.	Green (16, 23′, m)	do	Do.
Acetic acid, 99 percent, undiluted.	Yellow (16, 21′, i); finally colorless.	Dissolving or emulsifying.	No change.
Acetic acid, diluted to 50 percent.	do	Emulsifying	Do.
Ammonium hydroxide, specific gravity 0.9, undiluted.	Yellow (5, 25, —)	Rapidly emulsifying	Do.
Ammonium hydroxide, diluted to 83 percent.	do	do	Do.
Ammonium hydroxide, diluted to 63 percent.	do	do	Do.
Ammonium hydroxide, diluted to 50 percent.	do	do	Do
Potassium hydroxide, 10 percent.	Yellow (4, 23, —)	Dissolving	Do.
Potassium hydroxide, 5 percent.	do	Emulsifying or dissolving.	Do.
Phloroglucinol, alcoholic solution.	No change	Slightly dissolving	Do.
Alcohol and hydrochloric acid	Green (16, 23′, k)	Dissolving	Do.
Phloroglucinol and 50 percent hydrochloric acid.	Red violet (37, 65″ —).	do	No change; after heating, red (12, 69, f).
Naphthoresorcinol and 50 percent hydrochloric acid.	Violet (36, 57″, m)	Emulsifying and dissolving.	Do.
Orcinol and hydrochloric acid (Mathews (*2a*)).	Red (11, 65, k) after heating for 15 minutes.		Do.

[1] In parentheses are given first the plate number, second the color or hue number, and third the tone (Ridgway (*5*)).

Upon the addition of strong nitric acid the tissues of the embryo turned yellow (30, 23″, f) probably as a result of the presence of protein. The glands showed no changes, except sometimes a yellow color or emulsification.

With acetic acid the contents of the glands slowly but almost completely disappeared, evidently going into molecular or colloidal solutions. The contents of some of the glands entirely disappeared, but portions of others remained. The tissues of the embryo were unchanged in appearance.

TESTS FOR PENTOSANS

After the action of acids on the seeds had been determined, it was considered desirable to repeat the tests for pentosans previously reported (*4*) in order to check the interpretations that were made. Orcinol and phloroglucinol when used with hydrochloric acid are regularly employed in testing for pentosans. At least one acid, sulphuric, alone caused changes in the glands slightly resembling pentosan reactions obtained with phloroglucinol and hydrochloric acid or orcinol and hydrochloric acid. However, the results of the tests show that these changes were neither brought about in the glands by the use of hydrochloric acid, phloroglucinol, orcinol, naphthoresorcinol, or alcohol (the solvent used for phloroglucinol, orcinol, and naphthoresorcinol) alone, nor by any combination of them except phloroglucinol, orcinol, or naphthoresorcinol, used with the acid in the customary way of testing for pentosans. Therefore the phloroglucinol-hydrochloric acid test for pentosans performed in the customary way is applicable here when used cautiously and when the results are correctly interpreted.

The glands of many cottonseeds are red or purple before treatment, and if color alone following treatment were made the criterion of reaction, some difficulty would be experienced in determining whether results were positive or negative. For this reason the glands were always observed before any reagent was added.

After being treated with phloroglucinol and 50 percent hydrochloric acid and heated, the glands were invariably purple or red violet (37, 65″, —). This color also appeared if the preparations were allowed to stand for a few minutes at room temperature. When hydrochloric acid of full commercial strength instead of 50 percent was used, the same color occurred without heating or standing. Generally, lower concentrations of acid required more heat to bring about the reaction. With this treatment emulsification of the unbroken glands began around the periphery and slowly proceeded toward the center until the entire mass was affected. The contents did not go entirely into molecular solution as was shown by the persistence of visible particles that streamed from the glands.

Orcinol was substituted for phloroglucinol in this test, and a red color (11, 65, k) resulted. To obtain a positive test with this reagent, more heating was required than when phloroglucinol was used. When preparations were heated to the boiling point and allowed to stand for about 15 minutes, consistently positive results were obtained.

Naphthoresorcinol (in 70-percent alcoholic solution) and hydrochloric acid used in the customary way of testing for pentosans gave a violet (36, 57″, m) color in the glands. This reaction also occurred in the lignified structures. This test is considered to be a confirmation of the phloroglucinol-hydrochloric acid test, for naphthoresorcinol is a phenolic compound related to phloroglucinol.

Emphasis should be placed upon the fact that the occasional red color caused by hydrochloric acid alone, already described in this paper, did not occur in the glands but rather in the tissues of the embryo. The phloroglucinol-hydrochloric acid test when applied with heat sometimes was found to bring about a red coloration in the tissues, but since hydrochloric acid alone causes this change, there is no reason to consider it a pentosan reaction.

DISCUSSION

In exposed glands of the cotton plant the acids produced red colors and caused a certain amount of emulsification, while bases produced blue colors. These color reactions are interpreted as the result of the presence of an anthocyan pigment. Sulphuric was the only acid that produced a red color resembling pentosan reactions in the glands of the embryo, and this reaction evidently was caused by some substance other than pentosans which has the property of producing a red color with this treatment. The identity of this substance was not determined in the present study, but Marchlewski (*2*) stated that gossypol has this property, and later Withers and Carruth (*8*) and Stanford and Viehoever (*6*) considered gossypol to be a constituent of the glands of the cottonseed.

There are several indications that anthocyan pigments are absent, or almost absent, from the embryo. Sulphuric acid gave a red color reaction in these glands which bore only a slight resemblance to the reaction of an anthocyan pigment. Anthocyan pigments would not be expected in the seed for they do not develop in the absence of light. Further, no report is known to the writers of anthocyan in the cottonseed, whereas there are many reports of their occurrence in organs exposed to light.

Van Zwaluwenburg and Schlotterbeck (*7*) believed the component of the unexposed glands that gives the red color with phloroglucinol and hydrochloric acid to be a pigment, but they offered no suggestion as to the chemical nature of the pigment. It is improbable that any plant pigment would give a red color reaction with the phloroglucinol-hydrochloric acid test and not with hydrochloric, nitric, or acetic acid alone.

Hydrochloric acid alone sometimes produced a red color in the tissues of the embryo but not in the glands. The phloroglucinol-hydrochloric acid test for pentosans is therefore not thoroughly applicable to the extraglandular tissues of the embryo. No reaction in the glands of the cottonseed is recorded in these tests that would interfere with the phloroglucinol-hydrochloric acid test for pentosans. The only reagent that gave the exact change here regarded as a pentosan reaction was phloroglucinol or some generally recognized substitute for it, used with hydrochloric acid.

The facts point to the conclusion that either the cotton embryo contains no pentosans in any of its parts or that the glands of the cotton embryo do contain pentosans. Neither of these conditions seems to be impossible for the reasons given in the next two paragraphs.

Many investigators have reported pentosans or pentoses in the cotton embryo; but in most instances their tests have depended upon the production of furfural from the supposed pentosan and the subsequent reaction of this furfural with phloroglucinol. It is well known that many substances other than pentosans may be converted into furfural by acids. Common examples of these are found among the celluloses and lignocelluloses, which are hydrolyzable to hexoses. Hexoses are further convertible into hydroxymethylfurfuraldehyde, which, with phloroglucinol, may give reactions similar to those obtained from pentosans. Reeves and Valle (*4*) showed that the cell walls of the embryo and endosperm of the cottonseed are composed chiefly of cellulose, and the fringe tissue has long been known to be

composed largely of lignocellulose. The latter tissue is usually included with the embryo in making macrochemical analyses. Such compounds as glucuronic and galacturonic acids also produce furfural when treated with hydrochloric acid. Nucleoproteins and nucleic acid contain a pentose group, ribose. Nucleic acid, on account of this ribose group, gives the ordinary phloroglucinol-hydrochloric acid tests for pentosans (*3*). Jones and Csonka (*1*) reported a pentose protein that contained 16.57 percent pentose in the cotton embryo, but nevertheless concluded that it was not a nucleic acid.

On the other hand, there is reason to doubt that any of the compounds mentioned above, other than pentoses or pentosans, caused the color reaction described in the present investigation. Hydroxymethylfurfuraldehyde, which is derived from the hexosans and which gives furfural reactions, tends to decompose into laevulinic and formic acids when heated with hydrochloric acid. The hexoses, or their condensation products, would therefore give the phloroglucinol-hydrochloric acid reaction only slightly or not at all. Further, the component of the cottonseed giving this reaction was easily emulsified in water. It could not therefore be cellulose or lignocellulose. Such compounds as nucleic acid or nucleoproteins of the embryo would be expected to occur in the tissues rather than in the glands, because they are most frequently localized in the nuclei of cells. The only component of the cotton embryo that gave a test for pentosans was located in the glands.

It is not improbable that the component of the cotton embryo that usually has been regarded as a pentosan is a pentose combined with another compound. Resins often have been reported to occur in the glands, and the presence of pentosans in addition suggested some one of the substances called gum resins. But whatever the exact nature of the substance may be, all the evidence indicates that the pentosans, as well as the resins, are contained in the glands. The microchemical tests reported here are typical pentosan reactions except that when the tests are made with phloroglucinol and hydrochloric acid the reactions occur within a few minutes in a warm room without additional heat. It is probable, therefore, that the component of the glands giving the reactions with phloroglucinol, orcinol, and naphthoresorcinol used with hydrochloric acid, as recorded here, is a pentosan or a pentose.

SUMMARY AND CONCLUSIONS

Microchemical tests were made upon resin glands of the cotton embryo with the view of determining whether pentosans are localized in them.

Since the phloroglucinol-hydrochloric acid test, which is the best of the known microchemical tests for pentosans, is not specific; and since numerous color changes were caused by other reagents on various parts of the cotton plant, a number of supplementary tests were made in order to determine the applicability of the phloroglucinol-hydrochloric acid test.

In spite of the difficulties encountered in interpreting the reactions for pentosans, the phloroglucinol-hydrochloric acid test used according to standard procedure was found to cause a change in the glands of the embryo that is acceptable as a pentosan reaction. This test was verified with orcinol and with naphthoresorcinol as substitutes for

phloroglucinol. No other reagent produced a change in the glands which closely resembled that caused by the standard pentosan reagents.

The tests used for pentosans in this investigation cannot be successfully applied to the resin glands of the leaves on account of the presence of pigments which mask and otherwise confuse the results.

The glands were the only part of the embryo that gave a strong indication of the presence of pentosans.

The evidence obtained from this study strongly indicates that the resin glands are the chief containers of whatever pentosans are present in the cotton embryo.

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